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(54) Title: ACTIVE AGENT DELIVERY SYSTEMS AND METHODS FOR PROTECTING AND ADMINISTERING ACTIVE AGENTS

(57) Abstract: The present invention relates to active agent delivery systems and more specifically to compositions that comprise amino acids, as single amino acids or peptides, covalently attached to active agents and methods for administering conjugated active agent compositions.

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ACTIVE AGENT DELIVERY SYSTEMS AND METHODS FOR PROTECTING AND ADMINISTERING ACTIVE AGENTS

BACKGROUND OF THE INVENTION

(i) Field of the Invention

[001] The present invention relates to active agent delivery systems and, more specifically, to compositions that comprise peptides covalently attached to active agents and methods for administering conjugated active agent compositions.

(ii) Background of the Invention

[002] Active agent delivery systems are often critical for the safe effective administration of a biologically active agent (active agent). Perhaps the importance of these systems is best realized when patient compliance and consistent dosing are taken under consideration. For instance, reducing the dosing requirement for a drug from four-times-a-day (QID) to a single dose per day would have significant value in terms of ensuring patient compliance. Increasing the stability of the active agent, will assure dosage reproducibility and perhaps may also reduce the number of dosages required. Furthermore, any benefit that modulated absorption can confer on an existing drug would certainly improve the safety of the drug. Finally, improving the absorption of drugs should have a significant impact on the safety and efficacy of the drug.

[003] Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the gastrointestinal (GI) tract, lack of the agent's permeability and transport across lipid bilayers. These systems respond, in part, to the physicochemical properties of the drug molecule itself. Physical constants describing specific physicochemical properties like lipophilicity (log P) and ionizability (pK_a) depend on molecular structure of the active agent. Some drugs are poorly absorbed because they are too hydrophilic and do not effectively cross the plasma membranes of cells. Others are too lipophilic and are insoluble in the intestinal lumen and cannot migrate to the mucosa lining. The entire digestion and absorption process is a complex sequence of events, some of which are closely interdependent. There should exist optimum physicochemical

properties by which active agent bioavailability is maximized. However, it is often difficult to optimize these properties without losing therapeutic efficacy.

[004] Optimization of a drug's bioavailability has many potential benefits. For patient convenience and enhanced compliance it is generally recognized that less frequent dosing is desirable. By extending the period through which the drug is absorbed, a longer duration of action per dose is expected. This will then lead to an overall improvement of dosing parameters such as taking a drug twice a day where it has previously required four times a day dosing. Many drugs are presently given at a once a day dosing frequency. Yet, not all of these drugs have pharmacokinetic properties that are suitable for dosing intervals of exactly twenty-four hours. Extending the period through which these drugs are absorbed would also be beneficial.

[005] One of the fundamental considerations in drug therapy involves the relationship between blood levels and therapeutic activity. For most drugs, it is of primary importance that serum levels remain between a minimally effective concentration and a potentially toxic level. In pharmacokinetic terms, the peaks and troughs of a drug's blood levels ideally fit well within the therapeutic window of serum concentrations. For certain therapeutic agents, this window is so narrow that dosage formulation becomes critical. Such is the case with the drug, digoxin, which is used to treat heart failure.

[006] Digoxin therapeutic blood levels include the range between 0.8 ng/mL (below which the desired effects may not be observed) and about 2 ng/mL (above which toxicity may occur). Among adults in whom clinical toxicity has been observed, two thirds have serum concentrations greater than 2 ng/mL. Furthermore, adverse reactions can increase dramatically with small increases above this maximum level. For example, digoxin-induced arrhythmias occur at 10%, 50%, and 90% incidences at serum drug levels of 1.7, 2.5 and 3.3 ng/mL, respectively.

[007] After the oral administration of digoxin, an effect will usually be evident in 1-2 hours with peak effects being observed between 4 and 6 hours. After a sufficient time, the concentration in plasma and the total body store is dependent on the single daily maintenance dose. It is critical that this dose be individualized

for each patient. Having a dosage form of digoxin that provides a more consistent serum level between doses is therefore useful.

[008] Another example of the benefit of more consistent dosing is provided by the ß-blocker atenolol. The duration of effects for this commonly used drug is usually assumed to be 24 hours. However, at the normal dose range of 25-100 mg given once a day, the effect may wear off hours before the next dose begins acting. For patients being treated for angina, hypertension, or for the prevention of a heart attack, this may be particularly risky. One alternative is to give a larger dose than is necessary in order to get the desired level of action when the serum levels are the lowest. But this may cause side effects related to excessive concentrations in the initial hours of the dosing interval. At these higher levels, atenolol loses its potential advantages. ß-1 selectivity and adverse reactions related to the blockade of ß-2 receptors become more significant.

[009] In the case of anti-HIV nucleoside drugs, metering the absorption of the drug into the bloodstream has sufficient benefit. Drugs like AZT, for example, depend on phosphorylation to occur after absorption and before uptake into a virus in order to be effective. In normal dosing, drug levels increase rapidly after absorption that the phosphorylation reaction pathways can become saturated. Furthermore, the rate of phosphorylation is dependent on serum concentrations. The reactions occur more rapidly when concentrations are lower. Therefore, nucleoside analogs which retain lower serum concentrations are more efficiently converted to active drugs than other rapidly absorbed anti-viral drugs.

[010] Whereas the toxicity of digoxin and atenolol can be viewed as extensions of their desired activities, toxicity associated with the statins, on the other hand, is seemingly unrelated to the therapeutic effect of the drug. The toxic side effects of statins include, amongst other things, liver problems and rhabdomyolysis. Although the exact cause of statin-induced rhabdomyolysis and liver toxicity is not well understood, they have been linked to potent liver enzymes. The therapeutic effect of the statins is a result of the down-regulation of one of the key enzymes responsible for cholesterol production. Statin overdosing, however, can cause the reduced synthesis of non-sterol products that are important for cell growth, in

addition to rhabdomyolysis. So with the statins, a case can be made that by modulating the absorption of the drug, the therapeutic effect can be obtained at lower doses thereby minimizing the risk of producing toxic side effects.

[011] Finally, increasing the absorption of an active agent can have a significant impact on its safety. Taking the example of statins, once more, statins are anywhere between 10 and 30 % absorbed and dosing is based on the average of this range so for patients that absorb 30% of the statins administered, deleterious side effects can occur. The risk of manifesting these side effects would be greatly diminished if the bioavailability of the drug were more predictable.

[012] In an attempt to address the need for improved bioavailability several drug release modulation technologies have been developed. Enteric coatings have been used as a protector of pharmaceuticals in the stomach and microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation.

[013] A wide range of pharmaceutical formulations purportedly provides sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. The microencapsulation of therapeutics and diagnostic agents is generally described for example in U.S. 5,238,714 to Wallace et al.

[014] While microencapsulation and enteric coating technologies impart enhanced stability and time-release properties to active agent substances these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix or degradation of the matrix, which is

highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

[015] Active agents have been covalently attached to the amino acid side chains of polypeptides as pendant groups. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. An example of a timed and targeted release pharmaceutical administered intravenously, intraperitoneally, or intra-arterially includes the linking of nitrogen mustard, via a stabilizing peptide spacer, to a macromolecular carrier, e.g. poly-[N^5-(2-hydroxylethyl)-L-glutamine] (PHEG) which has an improved half-life when attached to the carrier and stabilizing unit.

[016] Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid as a colon-specific drug delivery system, which is released by bacterial hydrolytic enzymes residing in the large intestines. The dexamethasone active agent was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Other examples include techniques for forming peptide-linked biotin, peptide-linked acridine, naphthylacetic acid bonds to LH-RH, and coumarinic acid cyclic bonds to opioid peptides.

[017] Several implantable drug delivery systems have utilized polypeptide attachment to drugs. An example includes the linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of a large polyglutamic acid polymeric carrier designed to biodegrade over long periods of time after implantation via injection or surgical procedures. Additionally, other large polymeric carriers incorporating drugs into their matrices are used as implants for the gradual release of drug. Examples of implant delivery systems are generally described in U.S. 4,356,166 to Peterson et al. and 4,976,962 to Bichon et al. Yet another technology combines the advantages of covalent drug attachment with

liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Further description can be found in WO 97/36616 and U.S. 5,851,536 to Yager et al.

[018] Other systems were designed for the delivery of cytotoxic agents with specific amino acid sequences so that the drug will not be cleaved until the conjugate comes into contact with specific enzymes or receptors. One such example is the binding of oligopeptides directed toward enzymatically active prostrate specific antigen (PSA), to a cytotoxic agent which also typically contains a protecting group to prevent premature release of the conjugate in the blood. Another system designed for delivery via injection relies on specific peptides directly linked to a polymeric carrier, which is, in turn, attached to the cytotoxic moiety to be internalized by the cell. In this case, the polymeric carrier, typically large, will not enter cells lacking receptors for the specific peptide attached. In another example, gastrin receptor directed tetragastrin and pentagastrin amides were attached to cytotoxic drug for testing *in vitro*. These systems are generally described in U.S. 5,948,750 to Garsky et al.; U.S. 5,087,616 Myers et al.; and Schmidt et al., Peptide Linked 1,3-Dialkyl-3-acyltriazenes: Garstrin Receptor Directed Antineoplastic Alkylating Agents, Journal of Medical Chemistry, Vol. 37, No. 22, pp. 3812-17 (1994).

[019] Several systems have been directed to the treatment of tumor cells. In one case, Daunorubicin bound poly-L-aspartic acid, delivered intravenously or intraperitoneally, demonstrated lower cytotoxic effect. In another example, Daunorubicin was covalently attached via a methylketone side-chain of the drug to both poly-L-aspartic acid and poly-L-lysine The conjugates were prepared for intravenous and intraperitoneal administration. The poly-L-lysine was found to be ineffective, while the poly-L-aspartic acid conjugate showed preferential tumor cell uptake. In another system, a highly substituted polypetide conjugated to the active agent was designed for introduction into blood vessels that further penetrated the interstitium of tumors through long chain lengths. Further discussion of delivery systems targeted at tumor cells are described in Zunino et al., Comparison of Antitumor Effects of Daunorubicin Covalently Linked to Poly-L-Amino Acid

Carriers, Eur. J. Cancer Clin. Oncol., Vol. 20, No. 3, pp. 421-425 (1984); Zunino et al., Anti-tumor Activity of Daunorubicin Linked to Poly-L-Aspartic Acid, Int. J. Cancer, 465-470 (1982); and 5,762,909 to Uzgiris.

[020] Several examples relate to the delivery of paclitaxel, an anti-cancer drug. One delivery system relies on the drug remaining attached to the conjugate for transport into the cell via a membrane transport system. In another example, the paclitaxel is conjugated to a high molecular weight polyglutamic acid, and was delivered via injection. Paclitaxel conjugates have also been used with implants, coatings and injectables. These systems are further described in U.S. Patent 6,306,993; Li et al. Complete Regression of Well-established Tumors Using a Novel Water-soluble Poly(L-Glutamic Acid)-Paclitaxel Conjugate, pp. 2404-2409; and U.S. 5,977,163 to Li et al.

[021] Delivery systems can be designed to utilize attachment to chemical moieties that are either specifically recognized by a specialized transporters or have enhanced adsorption into target cells through specific polypeptide sequence. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. Evidence suggests that hydrophilic compounds are absorbed through the intestinal epithelia more efficiently through these specialized transporters than by passive diffusion. Active transport mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. Facilitation of these transporters can also depend on some sort of specialized adjuvant, which can result in localized delivery of an active agent, increased absorption of the active agent or some combination of both. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Increased bioavailability was found when peptides were bound to modified bile acids. Kramer et al., Intestinal Absorption of Peptides by Coupling to Bile Acids, The Journal of Biological Chemistry, Vol. 269, No. 14, pp. 10621-627 (1994). The use of specific polypeptide sequences to increase absorption is discussed in the literature where attaching the

drug to a polypeptide chain enhances the drug's permeability into cells. For example, Paul Wender of Stanford University reported the use of polyarginine tags on cyclosporine and taxol to facilitate diffusion across cell membranes. The penetratin system provides another example. This class of peptides, about 16 residues long, was shown to enhance absorption of oligonucleotides and polypeptides.

[022] It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Due to the inherent instability of non-covalent bonds, the bond between the active agent and the microsphere will usually not withstand the vigorous conditions used to reduce the composition's particle size.

[023] Thus, there has been no pharmaceutical composition, heretofore reported, that incorporates the concept of attaching an active agent to a peptide or its pendant group with targeted delivery of the active agent into the general systemic circulation. Furthermore, there has been no pharmaceutical composition that teaches the release of the active agent from the peptide by enzymatic action in the gastro intestinal tract.

[024] The need still exists for an active agent delivery system that does not require that the active agent be released within specific cells (e.g. a tumor-promoting cell), but rather results in release of the active agent for general systemic delivery.

[025] The need further exists for an active agent delivery system that allows for the oral delivery of active agents that will survive in the stomach and allow for the release of the reference active agent in the small intestines, the brush border membrane, in the intestinal epithelial cells or by enzymes in the bloodstream. The present invention also addresses the need for an active agent delivery system that is able to deliver active agents as an active agent peptide conjugate so that the molecular mass and physiochemical properties of the conjugate can be readily

manipulated to achieve the desired release rate. The need still exists for an active agent delivery system that allows for the active agent to be released over a sustained period of time, which is convenient for patient dosing.

[026] There is a generally recognized need to have an active agent delivery system that reduces the daily dosing requirement and allows for time released or controlled released absorption of the composition. The present invention accomplishes this by extending the period during which an active agent is absorbed, and providing a longer duration of action per dose than is currently expected. This leads to an overall improvement of dosing parameters such as, for example, taking an active agent twice a day where it has previously required four times a day dosing. Alternatively, many active agents presently given at a once a day dosing frequency lack the pharmacokinetic properties suitable for dosing intervals of exactly, twelve or twenty-four hours. An extended period of active agent adsorption for the current single dose active agents still exists and would be beneficial.

[027] Therefore, the need still exists for a drug delivery system that enables the use of new molecular compositions, which can reduce the technical, regulatory, and financial risks associated with active agents while improving the performance of the active agent in terms of its absorption parameters, as described above, and stability. Further, the need exists for an active agent delivery system targeted to general systemic circulation wherein the release of the drug from the peptide can occur by enzymatic action on the peptide-drug conjugate in the bloodstream or by enzymatic action on the peptide-drug conjugate in the alimentary tract followed by absorption through the intestines or stomach.

SUMMARY OF THE INVENTION

[028] The present invention provides covalent attachment of active agents to a peptide. The invention may be distinguished from the above mentioned technologies by virtue of covalently attaching the active agent directly, which includes, for example, pharmaceutical drugs and nutrients, to the N-terminus, the C-terminus or to the side chain of an amino acid, an oligopeptide or a polypeptide, also referred to herein as a carrier peptide. In another embodiment, when the active

agent is itself an amino acid active agent, then the active agent may be part of the chain at either the C-terminus or N-terminus through a peptide bond, or interspersed in the polypeptide via peptide bonds on both sides of the active agent.

[029] In another embodiment, the peptide stabilizes the active agent, primarily in the stomach, through conformational protection. In this application, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, endigenous enzymes release the active ingredient for absorption by the body by hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces the second phase of the sustained release mechanism.

[030] In another embodiment, the invention provides a composition comprising a peptide and an active agent covalently attached to the peptide. Preferably, the peptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids (L or D isomers), or an isomer, analogue, or derivative thereof, (iii) a heteropolymer of two or more naturally occurring amino acids (L or D isomers), or an isomer, analogue, or derivative thereof, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

[031] The invention provides compositions comprising a carrier peptide and an active agent covalently attached to the carrier peptide. Preferably, the carrier peptide is (i) an amino acid, (ii) a dipeptide, (iii) a tripeptide, (iv) an oligopeptide, or (v) polypeptide. The carrier peptide may also be (i) a homopolymer of a naturally occurring amino acids, (ii) a heteropolymer of two or more naturally occurring amino acids, (iii) a homopolymer of a synthetic amino acid, (iv) a heteropolymer of two or more synthetic amino acids, or (v) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

[032] A further embodiment of the carrier and/or conjugate is that the unattached portion of the carrier/conjugate is in a free and unprotected state.

[033] In another embodiment, the invention further provides a composition comprising an amino acid, a dipeptide or a tripeptide with an active agent covalently

attached. Preferably, the amino acid, dipeptide or tripeptide are (i) one of the twenty naturally occurring amino acids (L or D isomers), or an isomer, analogue, or derivative thereof, (ii) two or more naturally occurring amino acids (L or D isomers), or an isomer, analogue, or derivative thereof, (iii) a synthetic amino acid, (iv) two or more synthetic amino acids or (v) one or more naturally occurring amino acids and one or more synthetic amino acids. Preferably synthetic amino acids with alkyl side chains are selected from alkyls of C1-C17 in length and more preferably from C1-C6 in length.

[034] In one embodiment the active agent conjugate is attached to a single amino acid which is either naturally occurring or a synthetic amino acid. In another embodiment the active agent conjugate is attached to a dipeptide or tripeptide, which could be any combination of the naturally occurring amino acids and synthetic amino acids. In another embodiment the amino acids are selected from L-amino acids for digestion by proteases.

[035] In another embodiment, the peptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. In another embodiment, the peptide can be prepared through a fermentation process of recombinant microorganisms followed by harvesting and purification of the appropriate peptide. Alternatively, if a specific sequence of amino acids is desired, an automated peptide synthesizer can be used to produce a peptide with specific physicochemical properties for specific performance characteristics.

[036] In a preferred embodiment, the active agent is an inorganic acid or a carboxylic acid and the carboxylate or the acid group is covalently attached to the N-terminus of the peptide. In another preferred embodiment, the active agent is a sulfonamide or an amine and the amino group is covalently attached to the C-terminus of the peptide. In another preferred embodiment, the active agent is an alcohol and the alcohol group is covalently attached to the C-terminus of the peptide.

[037] In another embodiment, the active agent is itself an amino acid and is preferably covalently interspersed in the peptide in a peptide-linked manner or

covalently attached to a side chain, the N-terminus or the C-terminus of the peptide. In this embodiment when the amino acid active agents are attached to the C-terminus or the N-terminus this results in the active agent being the end amino acid and is considered C-capped or N-capped, respectively.

[038] The active agent can be covalently attached to the side chains of the polypeptide using conventional techniques. In a preferred embodiment a carboxylic acid containing active agent can be attached to the amine or alcohol group of the peptide side chain to form an amide or ester, respectively. In another preferred embodiment an amine containing active agent can be attached to the carboxylate, carbamide or guanine group of the side chain to form an amide or a new guanine group. In yet another embodiment of the invention, linkers can be selected from the group of all chemical classes of compounds such that virtually any side chain of the peptide can be attached.

[039] In a preferred embodiment the side chain attachment of an active agent to the polypeptide the amino acids used in either homopolymers or heteropolymers are selected from glutamic acid, aspartic acid, serine, lysine, cysteine, threonine, asparagine, arginine, tyrosine, and glutamine. Preferred examples of peptides include, Leu-Ser, Leu-Glu, homopolymers of Glu and Leu, and heteropolymers of (Glu)n-Leu-Ser.

[040] In another embodiment, direct attachment of an active agent to the carrier peptide may not form a stable compound therefore the incorporation of a linker between the active agent and the peptide is required. The linker should have a functional pendant group, such as a carboxylate, an alcohol, thiol, oxime, hydraxone, hydrazide, or an amine group, to covalently attach to the carrier peptide. In one preferred embodiment, the active agent is an alcohol and the alcohol group is covalently attached to the N-terminus of the peptide via a linker. In another preferred embodiment the active agent is a ketone or an aldehyde, which is attached to a linker through the formation of a ketal or acetal, respectively, and the linker has a pendant group that is attached to the carrier peptide. In yet another preferred embodiment the active agent is an amide, an imide, an imidazole or a urea where the

nitrogen is attached to the linker and the pendant group of the linker is attached to the carrier peptide.

[041] The invention also provides a method for preparing a composition comprising a peptide and an active agent covalently attached to the peptide. The method comprises the steps of:

(a) attaching the active agent to a side chain (and/or N terminus and/or C terminus) of an amino acid to form an active agent/amino acid complex;

(b) forming an amino acid complex N-carboxyanhydride (NCA) or forming an active agent/amino acid complex NCA from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

[042] In a preferred embodiment, the active agent is a pharmaceutical agent or an adjuvant. In another preferred embodiment, steps (a) and (b) are repeated with a second active agent prior to step (c). When steps (a) and (b) are repeated with a second agent prior to step (c), the active agent and second active agent can be copolymerized in step (c). Step (b) can include an amino acid (e.g. Glycine, Alanine, etc.), without an active agent attached, such that the product in step (c) is a copolymer of the active agent/amino acid complex and an amino acid interspersed in a peptide-linked manner.

[043] In a further embodiment of the above method, the amino acid itself can be an active agent (e.g. Thyroxine or DOPA) such that combining the NCA of this bioactive amino acid NCA with other amino acid NCA's will produce a product in (c) of the bioactive amino acid interspersed in the peptide with the generic amino acid in a peptide-linked manner.

[044] Alternatively, the active agent/amino acid complex can serve as a synthetic module for solid-phase or solution-phase peptide synthesis. Here, the drug can be attached to the selected amino acid by the α-amino group, the α-carboxylate or side chain functionality. Using these adducts in resin mounted peptide synthesis allows greater control of peptide composition, degree of loading, and relative

positioning of the drug. Thus the uses of these modules represent a unique approach for incorporating specified drugs at specific locations within peptides.

[045] Thus it is a further embodiment of this invention to expand the scope of current peptide synthesis technology to include novel amino acids derived from side-chain modified amino acids. In addition, the N-terminus of an amino acid can be modified for N-capped drug-peptide conjugate. Similarly, the C-terminus of an amino acid can be derivatized with the drug to ultimately produce a C-capped peptide-drug conjugate.

[046] The present invention provides for the synthesis whereby an active agent is conjugated to an amino acid, a dipeptide, a tripeptide, an oligopeptide or a polypeptide. Another embodiment of the present invention is dosage form reliability and batch-to-batch reproducibility.

[047] In another embodiment the active agent delivery is targeted into general systemic circulation. The release of the active agent from the peptide can occur by enzymatic action on the peptide-active agent conjugate in the bloodstream or by enzymatic action on the peptide-active agent conjugate in the alimentary tract followed by absorption through the intestines or stomach by the regular route of entry.

[048] In another embodiment, the invention also provides a method for delivering an active agent to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a peptide and an active agent covalently attached to the peptide. In a preferred embodiment, the active agent is released from the composition by enzyme catalysis. In another preferred embodiment, the active agent is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release.

[049] In another preferred embodiment, the generic amino acid is glutamic acid and the side chain attached active agent/glutamic acid complex is released from the peptide upon hydrolysis of the peptide and then the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and

serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a thioether, a carbonate, an anhydride, an orthoester, a hydroxamic acid, a hydrazone, sulfonamide, sulfonic esters, other derivatives of sulfur, or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, an urea, or an acid functionality.

[050] The composition of the invention can also include one or more microencapsulating agents, adjuvants and pharmaceutically acceptable excipients. The active agent can be bound to the microencapsulating agent, the adjuvant or the pharmaceutically acceptable excipient through covalent, ionic, hydrophilic interactions or by some other non-covalent means. The microencapsulating agent can be selected from polyethylene glycol (PEG), amino acids, carbohydrates or salts. If it is desired to delay peptide digestion, the microencapsulating agents can be used to delay protein unfolding. In another embodiment, when an adjuvant is included in the composition, the adjuvant preferably imparts better absorption either through enhancing permeability of the intestinal or stomach membrane or activating an intestinal transporter.

[051] The intestinal wall is coated with a mucosa lining made primarily of mucin. Many reagents have been identified that can bind to mucin. In another embodiment, the present invention provides the unique capability of binding a mucin-binding adjuvant to the peptide/drug conjugate to bioadhere the entire complex to the intestinal wall. The intestinal wall is impregnated with receptors for various reagents including many of the vitamins such as vitamin K. Binding vitamin K, for example, to the peptide-active agent conjugate will retain the entire complex in the intestines for a much longer time. It is the further embodiment of the invention wherein the adjuvant can bioadhere to the mucosal lining of the intestine thereby lengthening the transit time of the drug-peptide conjugate in the gut and maximizing peptide digestion and thus drug bioavailability.

[052] In another preferred embodiment, the composition further comprises a microencapsulating agent and the active agent conjugate is released from the composition by swelling or dissolution of the microencapsulating agent followed by

diffusion of the active agent conjugate which must then be acted upon by enzymes to release the active agent. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the peptide and release of the adjuvant from the composition is controlled by the enzymatic action on the peptide. The adjuvant can be microencapsulated into a carrier peptide-active agent conjugate for biphasic release of active agent. In another preferred embodiment, the peptide-active agent conjugate can be microencapsulated wherein the peptide-active agent conjugate is released in a biphasic manner, first through physicochemical means, such as through solvation or swelling, and then the active agent is released from the peptide carrier by enzymatic action. In yet another preferred embodiment of the invention, the active agent can be covalently attached to the microencapsulating agent via a peptide bond where the active agent is released first by peptidase action followed by migration of the active agent out of the microencapsulating medium.

[053] It is another embodiment of the present invention that the active agents may be combined with peptides of varying amino acid content to impart specific physicochemical properties to the conjugate including, molecular weight, size, functional groups, pH sensitivity, solubility, three dimensional structure and digestibility in order to provide desired performance characteristics. Similarly, a variety of active agents may also be used with specific preferred peptides to impart specific performance characteristics. Significant advantages with respect to the stability, release and/or adsorption characteristics of the active agent that are imparted through the use of one or more of the 20 naturally occurring amino acids are manifest in the peptide physicochemical properties that impart specific stability, digestibility and release properties to the conjugates formed with active agents.

[054] In another embodiment of the invention is the concept that the amino acids that make up the carrier peptide is a tool set such that the carrier peptide can conform to the pharmacological demand and the chemical structure of the active agent such that maximum stability and optimal performance of the composition are achieved.

[055] In another preferred embodiment the amino acid chain length can be varied to suit different delivery criteria. For delivery with increased bioavailability,

the active agent may be attached to a single amino acid to eight amino acids, with the range of two to five amino acids being preferred. For modulated delivery or increased bioavailability of active agents, the preferred length of the oligopeptide is between two and 50 amino acids in length. For conformational protection, extended digestion time and sustained release, preferred amino acid lengths may be between 8 and 400 amino acids. In another embodiment, the conjugates of the present invention are also suited for both large and small molecule active agents. In another embodiment of the present invention, the carrier peptide controls the solubility of the active agent-peptide conjugate and is not dependant on the solubility of the active agent. Therefore, the mechanism of sustained or zero-order kinetics afforded by the conjugate-drug composition avoids irregularities of release and cumbersome formulations encountered with typical dissolution controlled sustained release methods.

[056] In another preferred embodiment, the active agent conjugates can incorporate adjuvants such that the compositions are designed to interact with specific receptors so that targeted delivery may be achieved. These compositions provide targeted delivery in all regions of the gut and at specific sites along the intestinal wall. In another preferred embodiment, the active agent is released as the reference active agent from the peptide conjugate prior to entry into a target cell. In another preferred embodiment, the specific amino acid sequences used are not targeted to specific cell receptors or designed for recognition by a specific genetic sequence. In a more preferred embodiment, the peptide carrier is designed for recognition and/or is not recognized by tumor promoting cells. In another preferred embodiment, the active agent delivery system does not require that the active agent be released within a specific cell or intracellularly. In a preferred embodiment the carrier and/or the conjugate does not result in specific recognition in the body. (e.g. by a cancer cell, by primers, for improving chemotactic activity, by sequence for a specific binding cite for serum proteins(e.g. kinins or eicosanoids).

[057] In another embodiment the active agent may be attached to an adjuvant recognized and taken up by an active transporter. In a more preferred example the active transporter is not the bile acid active transporter. In another

embodiment, the present invention does not require the attachment of the active agent to an adjuvant recognized and taken up by an active transporter for delivery.

[058] While microsphere/capsules may be used in combination with the compositions of the invention, the compositions are preferably not incorporated with microspheres/capsules and do not require further additives to improve sustained release.

[059] In another preferred embodiment the active agent is not a hormone, glutamine, methotrexate, daunorubicin, a trypsin-kallikrein inhibitor, insulin, calmodulin, calcitonin, naltrexone, L-dopa, interleukins, gonadoliberin, norethindrone, tolmetin, valacyclovir, taxol, a GABA analog, an L-aromatic amino acid decarboxylase inhibitor, a catechol O-methyl transferase inhibitor, a naturally occurring amino acid, a bis-(2-chloroethyl)amine containing nitrogen mustard, a polypeptide, a peptidomimetic derived from a linear oligopeptide with greater the three amino acids, an oligonucleotide, a cyclophane derivative, a diehtylenetriaminopentaacetate derivative, histamine, a steroid or silver sulfadiazine. In a preferred embodiment wherein the active agent is a peptidic active agent it is preferred the active agent is unmodified (e.g. the amino acid structure is not substituted).

[060] In a preferred embodiment the invention provides a carrier and active agent which are bound to each other but otherwise unmodified in structure. This embodiment may further be described as the carrier having a free carboxy and/or amine terminal and/or side chain groups other than the location of attachment for the active agent. In a more preferred embodiment the carrier, whether a single amino acid, dipeptide, tripeptide, oligopeptide or polypeptide is comprises only naturally occurring amino acids.

[061] In a preferred embodiment the carrier is not a protein transporter (e.g. histone, insulin, transferrin, IGF, albumin or prolactin), Ala, Gly, Phe-Gly, or Phe-Phe. In a preferred embodiment the carrier is also preferably not a amino acid copolymerized with a non-amino acid substitute such as PVP, a poly(alkylene oxide) amino acid copolymer, or an alkyloxycarbonyl (polyaspartate/polyglutamate) or an aryloxycarbonylmethyl (polyaspartate/polyglutamate).

[062] In a preferred embodiment neither the carrier or the conjugate are used for assay purification, binding studies or enzyme analysis.

[063] In another embodiment, the carrier peptide allows for multiple active agents to be attached. The conjugates provide the added benefit of allowing multiple attachments not only of active agents, but of active agents in combination with other active agents, or other modified molecules which can further modify delivery, enhance release, targeted delivery, and/or enhance adsorption. In a further embodiment, the conjugates may also be combined with adjuvants or be microencapsulated.

[064] In another embodiment the conjugates provide for a wide range of pharmaceutical applications including drug delivery, cell targeting, and enhanced biological responsiveness.

[065] In another embodiment, the invention can stabilize the active agent and prevent digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed or sustained release of the active agent. The sustained release can occur by virtue of the active agent being covalently attached to the peptide and/or through the additional covalent attachment of an adjuvant that bioadheres to the intestinal mucosa. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced either by virtue of being covalently attached to a peptide or through the synergistic effect of an added adjuvant.

[066] In another preferred embodiment, the composition of the invention is in the form of an ingestible tablet or capsule, an intravenous preparation, an intramuscular preparation, a subcutaneous preparation, a depot implant, a transdermal preparation, an oral suspension, a sublingual preparation, an intranasal preparation, inhalers, or anal suppositories. In another embodiment, the peptide is capable of releasing the active agent from the composition in a pH-dependent manner. In another preferred embodiment the active agent is prepared and/or administered through means other than implantation and/or injectibles. In a preferred embodiments the active agent conjugate is not bound to an immobilized carrier, rather it is designed for transport and transition through the digestive system.

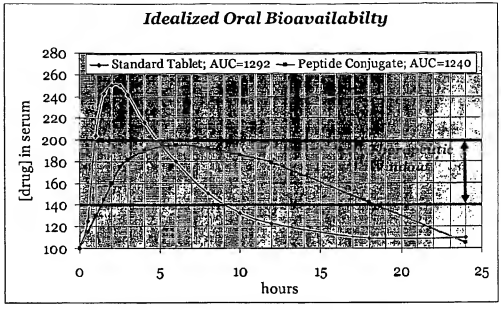
[067] Embodiments of the present invention preferably are not bound to an adjuvant recognized and/or taken up by active transporters. Preferably, the active agent conjugates of the present invention are not attached to active transporters, or antigenic agents such as receptor recognizing sequences found on cells and tumors. Preferably, the active agent conjugate of the present invention is not connected to or constitutes an implantable polymer, which would not biodegrade in less than 48 hours, preferably between 12 and 24 hours. The active agent conjugates of the present invention are preferably designed to release the active agent into the blood, after absorption from the gut, as the reference active agent.

[068] In another embodiment, following administration of the active agent conjugate by a method other than oral, first pass metabolism is prevented, by avoiding recognition of liver oxidation enzymes due to its peptidic structure.

[069] In another preferred embodiment the active agent is directly attached to the amino acid without the use of a linker.

[070] The invention also provides a method for protecting an active agent from degradation comprising covalently attaching the active agent to a peptide such that the peptide will impart conformational protection to the active agent.

[071] The invention also provides a method for controlling release of an active agent from a composition wherein the composition comprises a peptide, the method comprising covalently attaching the active agent to the peptide. It is a further embodiment of the invention that enhancement of the performance of active agents from a variety of chemical and therapeutic classes is accomplished by extending periods of sustained blood levels within the therapeutic window. For a drug where the standard formulation produces good bioavailability, the serum levels may peak too fast and too quickly for optimal clinical effect as illustrated below. Designing and synthesizing a specific peptide conjugate that releases the active agent upon digestion by intestinal enzymes mediates the release and absorption profile thus maintaining a comparable area under the curve while smoothing out active agent absorption over time.



[072] Conjugate prodrugs may afford sustained or extended release to the parent compound. Sustained release typically refers to shifting absorption toward slow first-order kinetics. Extended release typically refers to providing zero-order kinetics to the absorption of the compound. Bioavailability may also be affected by factors other than the absorption rate, such as first pass metabolism by the enterocytes and liver, and clearance rate by the kidneys. Mechanisms involving these factors require that the drug-conjugate is intact following absorption. The mechanism for timed release may be due to any or all of a number of factors. These factors include: 1) gradual enzymatic release of the parent drug by luminal digestive enzymes, 2) gradual release by surface associated enzymes of the intestinal mucosa, 3) gradual release by intacellular enzymes of the intestinal mucosal cells, 4) gradual release by serum enzymes, 5) conversion of a passive mechanism of absorption to an active mechanism of uptake, making drug absorption dependent on the Km for receptor binding as well as receptor density, 6) decreasing the solubility of the parent drug resulting in more gradual dissolution 7) an increase in solubility resulting in a larger amount of drug dissolved and therefore absorption over a longer period of time due to the increased amount available.

[073] The potential advantages of enzyme mediated release technology extend beyond the examples described above. For those active agents that can benefit from increased absorption, it is the embodiment of this invention that this effect is achieved by covalently bonding those active agents to one or more amino acids of the peptide and administering the drug to the patient as stated earlier. The invention also allows targeting to intestinal epithelial transport systems to facilitate absorption of active agents. Better bioavailability, in turn, may contribute to lower doses being needed. Thus it a further embodiment of the invention that by modulating the release and improving the bioavailability of an active agent in the manner described herein, reduced toxicity of the active agent can be achieved.

[074] It is another embodiment of this invention that attachment of an amino acid, oligopepetide, or polypeptide may enhance absorption/bioavailability of the parent drug by any number of mechanisms, including conversion of the parent drug to a polymer-drug conjugate such that the amino acid-prodrugs may be taken up by amino acid receptors and/or di- and tri-peptide receptors (PEPT transporters). This may also hold true for polymer drug conjugates since by products of enzymatic activity in the intestine may generate prodrugs with 1-3 amino acids attached. Moreover, it is possible that other receptors may be active in binding and uptake of the prodrugs. Adding an additional mechanism(s) for drug absorption may improve its bioavailability, particularly if the additional mechanism is more efficient than the mechanism for absorption of the parent drug. Many drugs are absorbed by passive diffusion. Therefore, attaching an amino acid to the compound may convert the mechanism of absorption from passive to active or in some cases a combination of active and passive uptake, since the prodrug may be gradually converted to the parent drug by enzymatic activity in the gut lumen.

[075] It is another embodiment of the invention that active agent efficiency is enhanced by lower active agent serum concentrations. It is yet another embodiment of the invention that conjugating a variety of active agents to a carrier peptide and, thereby sustaining the release and absorption of the active agent, would help achieve true once a day pharmacokinetics. In another embodiment of the invention, peaks and troughs can be ameliorated such as what could be achieved

with more constant atenolol levels, for example, following administration of a peptide-atenolol conjugate.

[076] In another embodiment of the present invention the amino acids used can make the conjugate more or less labile at certain pH's or temperatures depending on the delivery required. Further, in another embodiment, the selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will include glycine, alanine, valine, leucine, isoleucine, phenylalanine and tyrosine. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the peptide. In another embodiment, the amino acids with reactive side chains (e.g., glutamine, asparagines, glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attachment points with multiple active agents or adjuvants to the same carrier peptide. This embodiment is particularly useful to provide a synergistic effect between two or more active agents.

[077] In another embodiment, the peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. In another embodiment, the molecular weight of the carrier molecule can be controlled to provide reliable, reproducible and/or increased active agent loading.

[078] In another embodiment, the invention provides methods of testing the conjugates using Caco-2 cells.

[079] It is to be understood that both the foregoing general description and the following detailed description are exemplary, but not restrictive, of the invention. These and other aspects of the invention as well as various advantages and utilities will be more apparent with reference to the detailed description of the preferred embodiments and in the accompanying drawings.

[080] The present invention also addresses the need for non-protected active agents, which provide for ease of manufacture and delivery. The present invention also addresses the need for an active agent delivery system that is able to deliver active agents through the stomach as active agent peptide conjugates so that

the molecular mass and physiochemical properties of the conjugates can be readily manipulated to achieve the desired release rate. The present invention also addresses the need for an active agent delivery system that allows for the active agent to be released over an extended period of time, which is convenient for patient dosing. The present invention also addresses the need for an active agent delivery system that will provide protection through the stomach, but not require that the active agent be released within a specific cell or intracellularly.

[081] Embodiments of the present invention preferably do not produce an antigenic response or otherwise stimulate the immune system in the host. In another preferred embodiment the active agent conjugate attached to the carrier peptide is used to create an immune response when administered.

BRIEF DESCRIPTION OF THE DRAWINGS

[082] The invention is best understood from the following detailed description when read in connection with the accompanying drawing. Included in the drawing is the following figure:

Figure 1 describes the intramolecular transamination reaction of Glutamic acid;

Figure 2 illustrates the *in situ* digestion of Polythroid in intestinal epithelial cell cultures;

Figure 3 illustrates the improved adsorption of T4 from PolyT4 compared to T4 alone;

Figure 4 illustrates a decrease in the amount of Polythroid on the apical side over time (4hours) without intact Polythroid crossing the Caco-2 monolayer.

DETAILED DESCRIPTION OF THE INVENTION

[083] Throughout this application the use of "peptide" is meant to include a single amino acid, a dipeptide, a tripeptide, an oligopeptide, a polypeptide, or the carrier peptide. Oligopeptide is meant to include from 2 amino acids to 70 amino acids. Further, at times the invention is described as being an active agent attached to an amino acid, a dipeptide, a tripeptide, an oligopeptide, or polypeptide to

illustrate specific embodiments for the active agent conjugate. Preferred lengths of the conjugates and other preferred embodiments are described herein.

[084] Modulation is meant to include at least the affecting of change, or otherwise changing total absorption, rate of adsorption and/or target delivery. Sustained release is at least meant to include an increase in the amount of reference drug in the blood stream for a period up to 36 hours following delivery of the carrier peptide active agent composition as compared to the reference drug delivered alone. Sustained release may further be defined as release of the active agent into systemic blood circulation over a prolonged period of time relative to the release of the active agent in conventional formulations through similar delivery routes.

[085] The active agent is released from the composition by a pH-dependent unfolding of the carrier peptide or it is released from the composition by enzyme-catalysis. In a preferred embodiment, the active agent is released from the composition by a combination of a pH-dependent unfolding of the carrier peptide and enzyme-catalysis in a time-dependent manner . The active agent is released from the composition in a sustained release manner. In another preferred embodiment, the sustained release of the active agent from the composition has zero order, or nearly zero order, pharmacokinetics.

[086] The present invention provides several benefits for active agent delivery. First, the invention can stabilize the active agent and prevent digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed or sustained release of the active agent. The sustained release can occur by virtue of the active agent being covalently attached to the peptide and/or through the additional covalent attachment of an adjuvant that bioadheres to the intestinal mucosa. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced either by virtue of being covalently attached to a peptide or through the synergistic effect of an added adjuvant. The invention also allows targeted delivery of active agents to specific sites of action.

[087] A major portion of the enhanced performance imparted to active agents by the carrier peptide can be explained in terms of the composition's

structure. Proteins, oligopeptides, and polypeptides are polymers of amino acids that have primary, secondary, and tertiary structures. The secondary structure of the peptide is the local conformation of the peptide chain and consists of helices, pleated sheets, and turns. The peptide's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

[088] Peptides fold because of the dynamics associated between neighboring atoms on the peptide and solvent molecules. The thermodynamics of peptide folding and unfolding are defined by the free energy of a particular condition of the peptide that relies on a particular model. The process of peptide folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the peptide core occupy the same volume as they do in amino acid crystals. The folded peptide interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to peptide stability is the solid reference state.

[089] The major forces contributing to the thermodynamics of peptide folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy, and the hydrophobic effect. Considering peptide stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the peptide interior and exposing them to water. Comparing the energy of amino acid hydrolysis with peptide unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the peptide fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic peptide core. All of these forces combine and contribute to the overall stability of the folded peptide where the degree to which ideal packing occurs determines the degree of relative stability of the peptide. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

[090] Since it is likely that a hydrophobic active agent would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the active agent can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the peptide. The heat of hydration is a destabilization of a peptide. Typically, the folded state of a peptide is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, peptide unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a peptide is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, peptide conformation generally controls the rate and extent of deleterious chemical reactions.

[091] Conformational protection of active agents by peptides depends on the stability of the peptide's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for peptide unfolding.

[092] Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier peptide will be enriched in the amino acids that have bulky, lipophilic side chains. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the peptide.

[093] Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid, and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine, and arginine, ionize in the stomach and are neutral in an alkaline environment.

[094] Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking, and hydrogen bonding can all be used to select the optimum amino acid sequence for a desired performance parameter. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

[095] Variable molecular weights of the carrier peptide can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the peptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first phase of the release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier peptide.

[096] Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent. The enzymes encountered in the lumen of the intestines, on the intestinal cell surface, and within the cells lining the intestine may completely remove the drug from the carrier peptide before it reaches the bloodstream. Accordingly, concerns about the safety of the novel composition are eliminated

[097] Dextran has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has at least two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

[098] As a practical example, the Table 1 below lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

- Table 1 -

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176

Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

[100] Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a peptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

[101] In one embodiment the active agent is attached to a peptide that ranges between a single amino acid and 450 amino acids in length. In another embodiment two to 50 amino acids are preferred, with the range of one to 12 amino acids being more preferred, and one to 8 amino acids being most preferred. In another embodiment the number of amino acids is selected from 1, 2, 3, 4, 5, 6, or 7 amino acids. In another embodiment of the invention the molecular weight of the carrier portion of the conjugate is below about 2,500, more preferably below about 1,000 and most preferably below about 500.

[102] In another embodiment the active agent conjugate is a dimer, of an active agent and a single amino acid. In another embodiment the active agent conjugate is attached to a dipeptide or tripeptide.

[103] Compositions of the invention comprise four essential types of attachment. These types of attachment are termed: C-capped, N-capped, side-chain attached, and interspersed. C-capped comprises the covalent attachment of an active

agent to the C-terminus of a peptide either directly or through a linker. N-capped comprises the covalent attachment of an active agent to the N-terminus of a peptide either directly or through a linker. Side-chain attachment comprises the covalent attachment of an active agent to the functional sidechain of a peptide either directly or through a linker. Interspersed comprises the attachment of active agents which themselves are amino acids. In this case the active agent would constitute a portion of the amino acid chain. Interspersed is herein meant to include the amino acid active agent (drug) being at the C-terminus, N-terminus, or interspersed throughout the peptide. When amino acid active agents are attached to the C-terminus or the N-terminus this results in the active agent being the end amino and is considered C-capped or N-capped respectively. Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired. The present invention also envisions the use of multiple active agents or multiple attachment sites of active agents along a peptide chain. Further embodiments of the invention will become clear from the following disclosure.

[104] The alcohol, amine or carboxylic acid group of the active agent is covalently attached to the N-terminus, the C-terminus or the side chain of the peptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, one monomeric unit forming a new peptide bond in essence, adds a molecule to the end of the peptide.

[105] If the active agent is an amine, an alternate method of attaching the amine to the C-terminus of the peptide is to allow the amine to initiate polymerization of the amino acid NCA's. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. For example, when the active agent is an alcohol,

the alcohol can be converted into an alkylchloroformate with phosgene or triphosgene. This intermediate is then reacted with the N-terminus of the peptide carrier to produce an active agent peptide composition linked via a carbamate. The carbamate active ingredient may then be released from the peptide carrier by intestinal peptidases, amidases, or esterases.

[106] Alternatively, an alcohol active agent can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Figure 1.

[107] Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

[108] Alternatively, the alcohol can be added to the chloroformate of the side chain of polyserine as shown in section III of the Examples. The product is a carbonate, which upon hydrolysis of the peptide bond an intramolecular rearrangement occurs releasing the drug much of the same was as described above.

[109] If the active agent is a ketone or an aldehyde than a ketal is formed with a linker that has a pendant group suitable for attachment to the N-terminus, C-terminus or side chain of the peptide. For example, a ketal can be formed by the

reaction of methylribofuranoside or glucose with methylnaltrexone as shown in example of glucose reacting with methylnaltrexone. The remaining free hydroxyl from the sugar moiety can then be treated as an alcohol for attachment to the C-terminus or a suitable side chain of the carrier peptide.

[110] The invention also provides a method of imparting the same mechanism of action for other peptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these peptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-active agent dimer. The side-chain attached carrier peptide-active agent conjugate is preferably releases the active agent moiety through peptidase and not necessarily esterase activity. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

[111] If the active agent is an amide or an imide then the nitrogen of the active agent can add in a Michael fashion to the dihydopyran carboxylic acid alkyl ester as shown in section VII:D of the Examples. The R group can either be an electron-withdrawing group such that transesterification with the side chain of the peptide can occur or the R group can be part of the side chain of the peptide. The release of the active agent from the linker is imparted by hydrolysis of the peptide carboxylate bond followed by a concerted decarboxylation/elimination reaction.

[112] The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the peptide using known techniques. In the case where the active agent is an amino acid (e.g. Thyroxine, Triiodothyronine, DOPA, etc.) the active agent can be interspersed within the peptide chain in a peptide linked manner in addition to be covalently attached to the N-terminus, C-terminus or the side chains as described above. It is the preferred embodiment of the invention that the interspersed copolymer of the amino acid active agent and neutral amino acid be produced by polymerizing a mixture of the respective amino acid NCA's.

[113] The composition of the invention comprises a peptide and an active agent covalently attached to the peptide. Examples of active agents that may be used with the present invention include, but are not limited to, those active agents listed in Table 2, either alone or in combination with other agents contained within Table 2. As one of skill in the art would readily understand, the active agents listed within Table 2 may exist in modified form to facilitate bioavailability and/or activity (e.g., a Sodium salt, halide-containing derivatives or HCl forms of an active agent listed in Table 2). Accordingly, the invention encompasses variants (i.e., salts, halide derivatives, HCl forms) of the active agents listed in Table 2.

- Table 2 -

Abacavir Sulfate
Abarelix
Acarbose
ACE neural peptidase inhibitor
Acetaminophen
Acetaminophen and Hydrocodone Bitartrate
Acetaminophen; Codeine Phosphate
Acetaminophen; Propoxyphene Napsylate
Acetylsalicylic Acid
Acitretin
Activated Protein C
Acyclovir
Adefovir Dipivoxil
Adenosine
Adenosine A1 receptor antagonist
Adrenocorticotrophic Hormone
AGE crosslink breaker
Agi 1067
Albuterol
Alendronate Sodium
Allopurinal
Alpha 1 Proteinase Inhibitor
Alprazalom
Alprostadil
Alt 711
Altinicline
Amifostine
Amiodarone
Amitriptyline HCL
Amlodipine Besylate
Amlodipine Besylate; Benazepril Hcl
Amoxicillin
Amoxicillin and Clarithromicin
Amoxicillin; Clavulanate Potassium
Amprenavir
Anagrelide Hydrochloride
Anaritide
Anastrozole
Angiotensin II antagonist
Antifungal agent
Antisense Oligonucleotide
Arginine
Aripiprazole
Aspirin, Carisoprodol And Codeine
Astemizole
Atenolol
Atorvastatin Calcium
Atovaquone
Atrial natriuretic peptide
Avasimibe
Azathioprene
Azelastine Hydrochloride
Azithromycin Dehydrate
Baclofen
Bcx Cw1812
Befloxatone
Benazepril Hydrochloride
Benzatropine Mesylate
Betamethasone
Bicalutamide
Bile acid transport inhibitor
Bisoprolol
Bisoprolol/Hydrochlorothiazide
Bleomycin
Bms Cw193884
Bosentan
Bpi 21
Bromocriptine
Bupropion Hydrochloride
Buspirone
Butorphanol Tartrate
Cabergoline
Caffeine
Calcitriol
Candesartan Cilexetil
Candoxatril
Capecitabine
Captopril
Carbamazepine
Carbapenem antibiotic
Carbidopa/Levodopa
Carboplatin
Carisoprodol
Carvedilol
Caspofungin
Ceb 925
Cefaclor
Cefadroxil; Cefadroxil Hemihydrate
Cefazolin Sodium
Cefdinir
Cefixime
Cefotaxime Sodium
Cefotetan Disodium
Cefoxitin Sodium
Cefpodoxime Proxetil
Cefprozil
Ceftazidime
Ceftibuten Dehydrate

Cefuroxime Axetil
Cefuroxime Sodium
Celecoxib
Cephalexin
Cerivastatin Sodium
Cetirizine Hydrochloride
D-Chiroinositol
Chlorazepate Depot
Chlordiazepoxide
Chloropheniramine and hydrocodone
Chlorpheniramine
Cholecystokinin antagonist
Cholinergic channel modulator
Chondroitin
Ciclesonide
Cilansetron
Cilastatin Sodium; Imipenem
Cilomilast
Cimetidine
Ciprofloxacin
Cisapride
Cisatracurium Besylate
Cisplatin
Citalopram Hydrobromide
Clarithromycin
Clomipramine
Clonazepam
Clonidine HCL
Clopidogrel Bisulfate
Clozapine
Codeine
Codeine and Guaifenesin
Codeine and Promethazine
Codeine, Guaifenesin and Pseudoephedrine
Codeine, Phenylephrine and Promethazine
Colestipol HCL
Conivaptan
Cyclobenzaprine HCL
Cyclophosphamide
Cyclosporine
Dalteparin Sodium
Dapitant
Desmopressin Acetate
Desogestrel; Ethinyl Estradiol
Dextroamphetamine Sulfate
Dextromethorphan
Diacetylmorphine
Diazepam
Diclofenac Sodium
Diclofenac Sodium, Misoprostol
Dicyclomine HCL
Didanosine
Digoxin
Dihydrocodeine
Dihydromorphine
Diltiazem Hydrochloride
Dipyridamole
Divalproex Sodium
D-Methylphenidate
Docetaxel
Dolasetron Mesylate Monohydrate
Donepezil Hydrochloride
Dopamine/D5W
Doxazosin
Doxorubicin Hydrochloride
Duloxetine
Dutasteride
Ecadotril
Ecopipam
Edodekin Alfa (Interleukin-12)
Efavirenz
Emivirine
Enalapril
Enapril Maleate, Hydrochlorothiazide
Endothelin A receptor antagonist
Eniluracil
Enoxaparin Sodium
Epoetin Alfa Recombinant
Eptifibatide
Ergotamine Tartrate
Erythromycin
Erythromycin/Sulfsx
Esatenolol
Esterified Estrogens; ,Methyltestosterone
Estrogens, Conjugated
Estrogens, Conjugated; Medroxyprogesterone Acetate
Estropipate
Etanercept
Ethinyl Estradiol/Norethindrone
Ethinyl Estradiol; Desogestrel
Ethinyl Estradiol; Levonorgestrel
Ethinyl Estradiol; Norethindrone
Ethinyl Estradiol; Norgestimate
Ethinyl Estradiol; Norgestrel
Ethylmorphine
Etidronate Disodium
Etodolac
Etoposide
Etoricoxib
Exendin-4
Famciclovir

Famotidine
Felodipine
Fenofibrate
Fenretinide
Fentanyl
Fexofenadine Hydrochloride
Filgrastim SD01
Finasteride
Flecainide Acetate
Fluconazole
Fludrocortisone Acetate
Flumazenil
Fluorouracil
Fluoxetine
Flutamide
Fluticasone
Fluvastatin
Fluvoxamine Maleate
Follitropin Alfa/Beta
Formoterol
Fosinopril
Fosphenytoin Sodium
Furosemide
Gabapentin
Ganaxolone
Ganciclovir
Gantofiban
Gastrin CW17 Immunogen
Gastroprokinetic compound
Gemcitabine Hydrochloride
Gemfibrozil
Gentamicin Isoton
Gepirone Hydrochloride
Glatiramer Acetate
Glimepiride
Glipizide
Glucagon HCL
Glucosamine
Glyburide
Goserelin
Granisetron Hydrochloride
Guaifenesin And Hydrocodone
Haloperidal
Heparin
Himatropine Methylbromide and
Hydrocodone Bitartrate
Humanized monoclonal antibody, hul124
Huperzine
Hydrochlorothiazid
Hydrochlorothiazide; Triamterene
Hydrocodone
Hydrocodone Bitartrate and
Phenylpropanolamine
Hydromorphone
Hydromorphone HCL
Hydroxychloroquine Sulfate
Ibuprofen
Ibuprofen and Hydrocodone
Idarubicin HCL
Ilodecakin
Ilomastat
Imiglucerase
Imipramine HCL
Indinavir Sulfate
Infliximab
Inositol
Inositol and D-Chiroinositol
Insulin
Insulin analogue
Interferon Alfacon-1
Interferon Beta-1a
Interleukin-2
Interleukin-12
Iodixanol
Iodothyronine
Iodothyronine and Thyroxine
Iopromide
Ioxaglate Meglumine; Ioxaglate Sodium
Ipratropium
Irbesartan
Irinotecan Hydrochloride
Isosorbide Dinitrate
Isotretinoin
Isradipine
Itasetron
Itraconazole
Kavalactone
Ketoconazole
Ketolide antibiotic
Ketoprofen
Ketorolac
Ketotifen
Labetalol HCL
Lamivudine
Lamivudine; Zidovudine
Lamotrigine
Lansoprazole
Leflunomide
Lesopitron
Leuprolide Acetate
Levocarnitine
Levocetirizine

Levofloxacin
Levothyroxine
Lfa3tip
Lintuzumab
Lipoxygenase inhibitor
Lisinopril and Hydrochlorothiazide
Loperamide HCL
Loracarbef
Loratadine
Lorazepam
Losartan Potassium
Losartan Potassium; Hydrochlorothiazide
Lovastatin
Lym 1
Macrophage colony stimulating factor
Marimastat
Mecasermin
Medroxyprogesterone Acetate
Mefloquine Hydrochloride
Megestrol Acetate
Melatonin
Mercaptopurine
Meropenem
Mesalamine
Mesna
Metaxalone
Metformin
Methyldihydromorphinone
Methylphenidate HCL
Methylprednisolone Acetate
Metolazone
Metoprolol Succinate
Metronidazole
Milrinone Lactate
Minocycline HCL
Mirtazapine
Misoprostol
Mitiglinide
Mitoxantrone Hydrochloride
Mivacurium Chloride
Modafinil
Moexepril Hydrochloride
Montelukast Sodium
Montelukast Sodium and Fexofenadine Hydrochloride
Morphine Sulfate
Mycophenolate Mofetil
Nabumetone
Nadolol
Naltrexone
Naproxen Sodium
Naratriptan Hydrochloride
Nefazodone Hydrochloride
Nelarabine
Nelfinavir Mesylate
Nesiritide
Neuraminidase Inhibitor
Nevirapine
Nifedipine
Nimodipine
Nisoldipine
Nitrofurantoin, Nitrofurantoin, Macrocrystalline
Nizatidine
Noradrenalin and dopamine reuptake inhibitor
Norastemizole
Norethindrone
Norfloxacin
Nortriptyline HCL
Octreotide Acetate
Ofloxacin
Olanzapine
Omeprazole
Ondansetron Hydrochloride
Oprelvekin
Orally active carbohydrate
Oral nonsteroidal antiestrogen
Orlistat
Orphenadrine Citrate
Oxaprozin
Oxazepam
Oxybutynin Chloride
Oxycodone HCL
Oxycodone and Acetaminophen
Oxycodone/APAP
Oxymorphone
Paclitaxel
Pagoclone
Palivizumab
Pamidronate Disodium
Paricalcitrol
Paroxetine Hydrochloride
Pemetrexed
Pemoline
Penicillin V
Pentosan Polysulfate Sodium
Pentoxifylline
Pergolide
Phenobarbital
Phenytoin Sodium
Phytoseterol
Pioglitazone Hydrochloride

Piperacillin Sodium
Pleconaril
Poloxamer CW188
Posaconazole
Potassium Channel Modulator
Pramipexole Dihydrochloride
Pravastatin Sodium
Prednisone
Pregabalin
Primidone
Prinomastat
Prochlorperazine Maleate
Promethazine HCL
Propofol
Propoxyphene-N/APAP
Propranolol HCL
Prourokinase
Pseudoephedrine
Quetiapine Fumarate
Quinapril Hydrochloride
Quinolone antibiotic
Rabeprazole Sodium
Raloxifine Hydrochloride
Ramipril
Ranitidine
Ranolazine Hydrochloride
Recombinant Hepatitis B Vaccine
Relaxin
Remacemide
Repaglinide
Repinotan
Ribavirin
Riluzole
Rimantadine HCL
Risperidone
Ritonavir
Rizatriptan Benxoate
Rocuronium Bromide
Rofecoxib
Ropinirole Hydrochloride
Rosiglitazone Maleate
Rotavirus Vaccine
Rubitecan
Sagramostim
Saquinavir
Saquinavir Mesylate
Satraplatin
Selegiline HCL
Sertraline Hydrochloride
Sevelamer Hydrochloride
Sevirumab
Sibutramine Hydrochloride
Sildenafil Citrate
Simvastatin
Sinapultide
Sitafloxacin
Sodium channel blocker
Soluble chimeric protein CTLA4Ig
Sotalol HCL
Sparfosic Acid
Spironolactone
Stavudine
Sumatriptan
Tabimorelin
Tamoxifen Citrate
Tamsulosin Hydrochloride
Temazepam
Tenofovir Disoproxil
Tepoxalin
Terazosin HCL
Terbinafine Hydrochloride
Terbutaline Sulfate
Teriparatide
Tetracycline
Thalidomide
Theophylline
Thiotepa
Thrombopoetin, TPO
Thymosin Alpha
Tiagabine Hydrochloride
Ticlopidine Hydrochloride
Tifacogin
Tirapazamine
Tirofiban Hydrochloride
Tizanidine Hydrochloride
Tobramycin Sulfate
Tolterodine Tartrate
Tomoxetine
Topiramate
Topotecan HCL
Toresemide
Tpa Analogue
Tramadol HCL
Trandolapril
Trastuzumab
Trazadone HCL
Triamterene/HCTZ
Troglitazone
Trovafloxacin Mesylate
Urokinase
Ursodiol
Valacyclovir Hydrochloride

Valdecoxib
Valproic Acid
Valsartan, Hydrochlorothiazide
Valspodar
Vancomycin HCL
Vecuronium Bromide
Venlafaxine Hydrochloride
Verapamil HCL
Vinorelbine Tartrate
Vitamin B12
Vitamin C
Voriconazole
Warfarin Sodium
Xaliproden
Zafirlukast
Zaleplon
Zenarestat
Zidovudine
Zolmitriptan
Zolpidem

[114] The present invention allows for the combination of different active agents with a variety of peptides to impart specific characteristics according to the desired solubility, pH or folding. Similarly, the variety of peptides may be used to impart specific physicochemical properties to produce specific performance characteristics. The present invention provides significant advantages with respect to the stability and release and/or adsorption characteristics of the active agent(s). The conjugates of the present invention are also suited for delivery of both large and small molecules.

[115] In another embodiment, the use of one or more of the 20 naturally occurring amino acids as individual amino acids, in oligopeptides, or in polypeptides impart specific stability, digestibility and release characteristics to the conjugates formed with active agents.

[116] In another embodiment, the active agent conjugates are designed to interact with specific indigenous enzymes so that targeted delivery may be achieved. These conjugates provide targeted delivery in all regions of the gut and at specific sites along the intestinal wall. In another preferred embodiment, the active agent conjugates can incorporate adjuvants such that the compositions are designed to interact with specific receptors so that targeted delivery may be achieved. These compositions provide targeted delivery in all regions of the gut and at specific sites along the intestinal wall. In another preferred embodiment, the active agent is released as the reference active agent from the peptide conjugate prior to entry into a target cell. In another preferred embodiment, the specific amino acid sequences used are not targeted to specific cell receptors or designed for recognition by a specific genetic sequence. In a more preferred embodiment, the peptide carrier is designed for recognition and/or is not recognized by tumor promoting cells. In another preferred embodiment, the active agent delivery system does not require that the active agent be released within a specific cell or intracellularly.

[117] In another embodiment, the active agent conjugate allows for multiple active agents to be attached. The conjugates provide the added benefit of allowing multiple attachment not only of active agents, but of active agents in combination with other active agents, or other modified molecules which can further

modify delivery, enhance release, target delivery, and/or enhance adsorption. By way of example, the conjugates may also be combined with adjuvants or can be microencapsulated.

[118] In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the brush border membrane (BBM); and bile acids, which have been attached to peptides to enhance absorption of the peptides.

[119] In another embodiment absorption may be improved by increasing the solubility of the parent drug through selective attachment of an amino acid, oligopeptide, or polypeptide. Increasing solubility results in an increase in the dissolution rate. Consequently, there is an increase in the total amount of drug that is available for absorption; since the drug must be in solution for absorption to occur, bioavailability is increased.

[120] In another embodiment the compositions provide for a wide range of pharmaceutical applications including active agent delivery, cell targeting, and enhanced biological responsiveness.

[121] The present invention provides several benefits for active agent delivery. First, the invention can stabilize the active agent and prevent digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed or sustained release of the active agent. The sustained release can occur by virtue of the active agent being covalently attached to the peptide and/or through the additional covalent attachment of an adjuvant that bioadheres to the intestinal mucosa. Furthermore, active agents can be combined to produce synergistic effects.

[122] Absorption of the active agent in the intestinal tract can be enhanced either by virtue of being covalently attached to a peptide or through the synergistic effect of an added adjuvant. In a preferred embodiment of the invention the

absorption of the active agent is increased due to its covalent attachment to a peptide, hereafter to be referred to as a transporter peptide, which is a specialized example of a carrier peptide. In a further embodiment, the transporter peptide activates a specific peptide transporter. In yet another embodiment the peptide transporter is either the PepT1 or the PepT2 transporters. In a preferred embodiment the transporter peptide contains two amino acids. In another preferred embodiment the transporter dipeptide is selected from the list of AlaSer, CysSer, AspSer, GluSer, PheSer, GlySer, HisSer, IleSer, LysSer, LeuSer, MetSer, AsnSer, ProSer, GlnSer, ArgSer, SerSer, ThrSer, ValSer, TrpSer, TyrSer.

[123] In another embodiment, the present invention does not require the attachment of the active agent to an adjuvant that recognizes or is taken up by an active transporter. The invention also allows targeted delivery of active agents to specifics sites of action.

[124] In another preferred embodiment the chain length of amino acid can be varied to suit different delivery criteria. In one embodiment, the present invention allows for the delivery of active agents with sustained release.

[125] The invention may further be characterized by the following embodiments wherein the active agent is released as the reference active agent from the amino acid conjugate prior to entry into the target cell for the active agent. In another preferred embodiment the active agent is prepared and/or administered through means other than implantation and/or injectibles. Embodiments of the present invention preferably do not produce and antigenic response or otherwise stimulate the immune system in the host.

[126] Another embodiment of the present invention, for compositions administered by methods outside the alimentary tract, the intact conjugate may be less susceptible to first pass metabolism by the enterocytes, including biotransformation by cytochrome P450 (CYP) 3A4 and efflux by transporter P-glycoprotein. Immunogenicity and metabolic effects are avoided through maintenance of the three dimensional structure of the composition, maintaining

blood levels below the threshold value for expression of these metabolic factors or some other means.

[127] In another preferred embodiment the active agent is directly attached to the amino acid without the use of a linker.

[128] To predict the absorption of orally delivered drugs monolayers of Caco-2 human intestinal epithelial cells are increasingly being used. Caco-2 cells are grown on the surface of collagen-coated wells in a 24 well format to form confluent monolayers that represent small segments of the intestine. The wells are removable and contain a top chamber representing the apical side (facing the lumen of the intestine) and a bottom chamber representing the basolateral side (site of serosal drug absorption). Testing the electrical resistance across the monolayer monitors the integrity of the epithelial barrier. Absorption of drugs can be studied by adding sample to the apical side and assaying the concentration of the drug in the basolateral chamber following incubation.

[129] The small intestine has an extremely large surface area covered with highly specialized epithelial cells that produce both extracellular and intracellular enzymes. The Caco-2 cells also release enzymes similar to the epithelial cells of the small intestine. There is not much precedent for using the Caco-2 cells as models for digesting synthetic peptides. However, drug release from peptides on the apical side of Caco-2 transwell monolayers can be measured. The copolymer of glutamic acid and thyroxine enhanced the absorption of thyroxine across the Caco-2 monolayers.

[130] In another embodiment, the invention provides methods of testing the conjugates using Caco-2 cells.

[131] The Table 3 provides a list of active agents that have been covalently attached to a peptide. The table also provides a list of typical areas of use for the active agent conjugate.

- Table 3: List of Active Agents and Peptide Conjugates -

Typical Use of Active Agent	Generic Name	Peptide
Cardiovascular	Atenolol	Glu
Cardiovascular	Furosemide	Glu, Ser
Cardiovascular	Lisinopril	Glu
Metabolic & Endocrinology	Tetraiodothyronine	D,E,F,G,I,K,L,M,S,T,V
Metabolic & Endocrinology	Triiodothyronine	Glu
Metabolic & Endocrinology	T4 and T3	Glu
GI & Coag	Metoclopramide	Glu
Antiviral	Acyclovir	Glu
Anti-Infective	Amoxicillin	Glu
Cardiovascular	Digoxin	Glu
Cardiovascular	Dipyridamole	Glu
Cardiovascular	Gemfibrozil	Lys
Cardiovascular	Losartan	Glu
Neurology	Divalproex	Lys
Neurology	Gabapentin	Glu
Neurology	Levo/Carbidopa	Glu
Neurology	Quetiapine	Glu
Neurology	Sertraline	Glu
Addiction Treatment	Naltrexone	E,K,S,ES,EW
Addiction Treatment	Methylnaltrexone	Glu
Pulmonary & Allergy	Fexofenidine	Glu
Rep & Urology	Tolteridine	Glu
Anti-Infective	Cephalexin	Glu
Anti-Infective	Ciprofloxacin	Glu
Anti-Infective	Mesalamine	Glu
Anti-Infective	Metronidazole	Glu
Anti-Infective	Prednisone	Glu
Anti-Infective	Raloxifene	Glu
Anti-Viral	Stavudine	Glu
Anti-Viral	Zalcitabine	Glu
Anti-Viral	Zidovudine	Glu

Typical Use of Active Agent	Generic Name	Peptide
Anti-Infective	Ibuprofen	Lys
Anti-Infective	Naproxen	Lys
Anti-Infective	Dexamethasone	Glu
OTC	Acetaminophen	Glu
Cardiovascular	Arginine	Arg
Cardiovascular	Atorvastatin	Glu
Cardiovascular	Pravastatin	Lys
Cardiovascular	Simvastatin	Glu
Anti-Infective	Azithromycin	Glu

A=Alanine, D=Aspartic Acid, E=Glutamic Acid, F=Phenyalanine, G=Glycine, I=Isoleucine, K=Lysine, M=Methionine, S=Serine, T=Threonine, V=Valine

[132] The compositions of the invention can be formulated in pharmaceutical compositions by combining the compound with a pharmaceutically acceptable excipient known in the art. The conjugates may be employed in powder or crystalline form, in liquid solution, or in suspension. The conjugates of the present invention may be administered by a variety of means, including but not limited to: topically, orally, parenterally by injection (intravenously, intramuscularly or subcutaneously), as a depot implant, an intranasal preparation, an inhaler, or as an anal suppository. The injectable compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain various formulating agents. Alternatively, the conjugate may be in powder form for reconstitution at the time of delivery with a suitable vehicle, such as sterile water. In injectable compositions, the carrier is typically comprised of sterile water, saline or another injectable liquid, e.g., peanut oil for intramuscular injections. Also, various buffering agents, preservatives and the like can be included. Topical applications may be formulated in carriers such as hydrophobic or hydrophilic bases to form ointments, creams, lotions, in aqueous, oleaginous or in dry diluents to form powders. Oral compositions may take such forms as tablets, capsules, oral suspensions and oral solutions. The oral compositions may utilize carriers such as conventional formulating agents, and may include sustained release properties as

well as rapid delivery forms. The dosage to be administered depends to a large extent upon the condition and size of the subject being treated, the route and frequency of administration. One embodiment of the methods of administration of the conjugates includes oral and parenteral methods, e.g., i.v. infusion, i.v. bolus and i.m. injection. In a further embodiment of the invention, the composition incorporates a microencapsulating agent. Preferably, the composition of the invention is in the form of an ingestible tablet or capsule, an implantable device, a skin patch, a sublingual preparation, a subcutaneous preparation, an intravenous preparation, an intraperitoneal preparation, an intramuscular preparation or an oral suspension. Most preferably the compositions are formulated for oral delivery.

[133] Throughout the applications the figures are meant to describe the general scheme of attaching active agents through different functional groups to a variety of peptide conjugates resulting in different embodiments of the present invention. One skilled in the art would recognize other reagents, conditions, and properties necessary to conjugate other active agents to other peptides from the schemes, which are meant to be non-limiting examples. The figures further represent the different embodiments of the present invention with regard to length of the active agent conjugate wherein the amino acid, dipeptide, tripeptide, oligopeptide and peptide active agent conjugates can be respectively represented by n=0 for an amino acid, and n ≥ for other peptide embodiments.

I N-Terminus Attachment of an Active Agents to a Peptide

[134] The N-terminus attachment of active agent to a peptide can be formed through a plurality of active agent functional groups. Non-limiting examples of active agent functional groups include an alcohol group, a carboxylic acid group, an amine group or other reactive substiuents. The preferred active agent attaching functionalities for N-terminus attachment to a peptide include carboxylic acids, ketones and aldehydes. When the attachment at the N-terminus is to be made with an alcohol or its equivalent, or an amine or its equivalent an insertion of a linker between the functional group and the active agent is typically required.

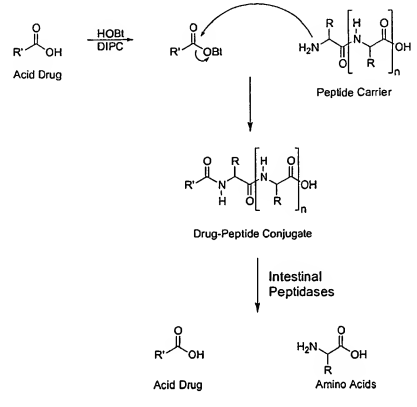
[135] Any amino acid may be used as the N-terminus of the peptide/active agent conjugate. Preferred amino acids for attachment include glutamic acid, aspartic acid, serine, and lysine.

[136] Specific examples of an active agent attached to the N-terminus below are meant for example purposes only and are not meant to limit the invention to either specific active agents, amino acids or combinations thereof. Preferred drugs for N-terminus attachment typically provide a carboxylic acid or an inorganic functional group for conjugation. By way of example, ibuprofen, furosemide, gemfibrozil, naproxen may be attached to the N-terminus.

[137] The below schemes depict methods of attaching active agents to the N-terminus. These figures and procedures describe the general scheme of attaching active agents to the N-terminus of a peptide.

(i) General Mechanism and Description of Conjugation of Acid Drug Attachment to the N-Terminus

- Acid Drug/N-Terminus Scheme -

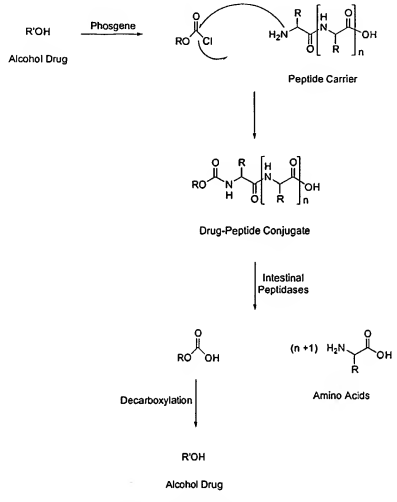


R'=Radical moiety attached to acid functionality on drug
R=Side chain of amino acid or peptide
HOBt=Hydroxybenzotriazole
DIPC=Diisopropylcarbodiimide

[138] As depicted an acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0 ^{o}C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

(ii) General Mechanism and Description of Conjugation of Alcohol Drug Attachment to the N-Terminus

- Alcohol Drug/N-Terminus Scheme -



R' = Radical moiety attached to alcohol functionality on drug
R = Side chain of amino acid or peptide

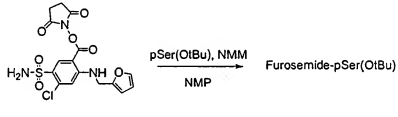
[139] As depicted above the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours.

The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

[140] Other solvents, activating agents, co-catalysts and bases can be used. Examples of other solvents include dimethylsulfoxide (DMSO), ethers such as tetrahydrofuran(THF) or chlorinated solvents such as chloroform ($CHCl_3$). Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another co-catalyst is N-hydroxysuccinimide (NHS). Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine (Et_3N) or tributylamine.

I:A – Example: Attachment of Furosemide via Carboxylic Acid to the N-terminus of Poly(Serine)

[141] The below example describes the attachment of an amino acid active agent to the N-terminus of a peptide. The example uses polySer attached to Furosemide.



Reagents	Weight	MW	Molar Equivalents
1. Furosemide-OSu	0.197g	427.7	1
1. pSer(OtBu)	0.330g	143 per residue	5
1. N-methyl morpholine	0.51 mL	101	10
1. N-methyl pyrrolidinone	5 mL		
2. 95% TFA/H_2O	20 mL		

[142] To a solution of pSer(OtBu) in N-methyl pyrrolidinone (NMP) was added Furosemide-OSu and N-methyl morpholine (NMM). The reaction was stirred overnight at room temperature. Solid material remained so reaction was stirred

overnight at 60°C. After cooling, reaction was placed in water (50 mL), solid was collected by filtration and solid was dried (0.480 g, 86% yield).

[143] Deprotection of pSer(OtBu) proceeded by adding 95%TFA/H_2O to the above material. The resulting dark solution was stirred overnight. Solvent was then removed, $NaHCO_3$ (saturated solution) added and the crude product was purified using ultrafiltration (YM1) to obtain Furosemide-pSer (0.101g) as a dark green solid.

I:B – Example: Attachment of Enalipril via Carboxylic Acid to the N-terminus of GluGlu.

Synthesis of Enalipril-Glu-Glu

[144] To enalipril maleate (0.200 g, 406 umol), hydroxybenzotriazole (0.164 g, 1217 umol), Glu(OtBu)Glu(OtBu)OtBu (0.560 g, 1217 umol) and diisopropylethylamine (0.210 ml, 1217 umol) in 7 ml dry DMF was added O-(1H-benzotriazol-1yl)N,N,N',N' tetramethyluronium hexafluorophosphate (0.461 g, 1217 umol). After stirring for 16 h under Argon, 40 ml of saturated NaCl (aq) was added to the black solution. The reaction was extracted 2x10 ml EtOAc (dark colored stayed with the organics). The organics were dried with anhyd. $MgSO_4$, filtered and the solvent removed by rotary evaporation. The residue was purified by flash chromatography (SiO_2 1:0-60:1-40:1-30:1-20:1-10:1) to provide Enalipril-Glu(OtBu)Glu(OtBu)OtBu as a yellowish gum (0.231 g, 54%): R_f 0.43 (9:1 $CHCl_3$:MeOH + 1 drop HOAc); ^{1}H-NMR (DMSO/$CDCl_3$) 7.13 (m, 5H), 4.43-4.09 (m, 5H), 2.20 (m, 7H), 1.97-1.80 (m, 13H), 1.35 (m, 27H), 1.22 (m, 6H); ESMS 803.

[145] Enalipril-Glu(OtBu)Glu(OtBu)OtBu (0.172 g, 214 umol) was stirred for 7.5 h with 9.5 ml trifluoroacetic acid and 0.5 ml H2O. The solvent was removed by rotary evaporation and the residue dried in vacuum. 1H-NMR (DMSO) 7.13 (m, 5H), 4.43-4.09 (m, 5H), 2.20 (m, 7H), 1.97-1.80 (m, 13H), 1.42 (m, 3H), 1.22 (m, 3H); FABMS calc 635.293, ob 635.490.

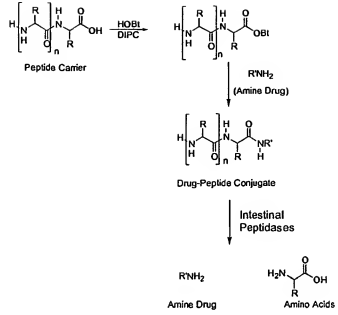
II C-Terminus Attachment of Active Agents to a Peptide

[146] The C-terminus attachment of an active agent to a peptide can be formed through a plurality of active agent functional groups. The functional groups include amines and their equivalents and alcohols and their equivalents. While any amino acid may be used to connect the active agent to the C-terminus, glutamic acid, aspartic acid, serine and lysine are preferred amino acids. Preferred active agents for C-terminus attachment are active agents with alcohol and amino functional groups. More preferred active agents include atenolol, metropolol, propanolol, methylphenidate and sertraline.

[147] The below scheme depicts methods of attaching active agents to the C-terminus. One skilled in the art would recognize other reagents, conditions, and properties necessary to conjugate other active agents from the schemes, which are meant to be non-limiting examples.

[148] This figure and procedure describe the general scheme of attaching an amine active agent to the C-terminus of a peptide. In the below scheme the peptide carrier can be dissolved in DMF under nitrogen and cooled to 0 ^{o}C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

- Amine Drug/C-Terminus Scheme -

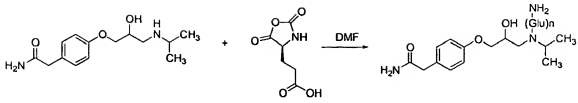


R'=Radical moiety attached to acid functionality on drug
R=Side chain of amino acid or peptide
HOBt=Hydroxybenzotriazole
DIPC=Diisopropylcarbodiimide

[149] Specific examples of active agent attached to the C-terminus below are meant for example purposes only and are not meant to limit the invention to either specific active agents, amino acids or combinations thereof.

II:A – Example: Amine-Initiated Polymerization of L-Glutamic Acid NCA

[150] This example can be used to generically describe the process of an amine active agent initiating polymerization of an amino acid NCA. The following procedure was successfully used to synthesize the polyglutamic acid conjugate of atenolol through its amine functionality. It should also be noted that atenolol is also an alcohol active agent and can also initiate polymerization of amino acid NCA's. This procedure can readily be applied to other amine drugs described herein.



- Table 4 -

MW (g.mol^{-1})	266.3	173	1538 (n=10)
Mass (mg)	77	500	446 = 100%
mmoles	0.29	2.89	0.29
Equivalents	1	10	1

[151] Table 4 describes the relative proportions that would be used in a typical atenolol preparation. Other amine drugs that would be used to initiate polymerization of an amino acid NCA would be expected to utilize similar proportions.

[152] The procedure is further described below, although those skilled in the art would recognize other solvents, proportions and reaction conditions that could be utilzed to achieve the desired results. DMF is dimethylformamide, anhydrous, and was purchased from Aldrich. The glassware was oven-dried prior to use. Glu-NCA (500 mg, 2.89 mmoles) was dissolved in 4 mL of DMF and stirred under argon in a 15 mL round bottom flask equipped with a gas inlet tube. Atenolol, dissolved in 1 mL of DMF, was added to this solution of Glu-NCA and allowed to stir at room temperature for 72 h. In general, the reactions can be run until there is no free amine initiator by Thin Layer Chromatography (TLC). For this reaction, TLC was run using silica plates and eluting with 20% methanol in ethyl acetate. The reaction was quenched by pouring into 20 mL of 10% sodium bicarbonate in water (pH = 8). The water was washed with 3 x 20 mL of methylene chloride and 3 x 20 mL of ethyl acetate. Combined aqueous layers were brought to a pH of 6 with 6N hydrochloric acid (HCl) and reduced to a volume of about 20 mL by rotary evaporation.

[153] This solution was then cooled in the refrigerator for > 3 hours. To precipitate the polymeric product, the aqueous solution was then acidified to a pH of about 2 using 6N HCl and placed back in the refrigerator for 1-2 hours. The suspension was poured by portions into a 10 mL test tube and centrifuged for 15 minutes until the precipitate formed a solid pack at the bottom of the tube from which the water could be decanted. (At this point in the general procedure, it is preferable that the solid be filtered through a filter funnel and washed with acidic water. The centrifuge was used for atenolol because the solid was too thin to filter.) The solid was then resuspended in acidic water (pH about 2) and vortexed before being centrifuged again and the water decanted. This procedure was repeated once more for a total of three washes. The solid was then dried by high vacuum overnight yielding 262 mg (59%) of polymer. NMR analysis indicated that the Glu/Atenolol ratio was about 30/1.

II:B – Example: Preparation of $(Glu)_n$-Cephalexin via an Amine Bond

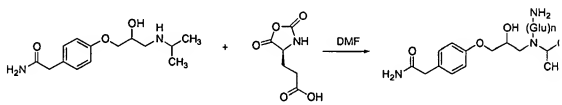
[154] The below example describes the attachment of an amino acid active agent to the C-terminus of a peptide. The example uses glutamic acid NCA to produce a polyGlu attached to Cephalexin. Cephalexin attached to a single amino acid may be produced via the below method if an excess amount of Cephalexin is added to the procedure.

[155] Glu(OtBu)NCA (1.000 g, 4.4 mmol) and Cephalexin•HCl (0.106 g, 0.3 mmol) were dissolved in anhydrous DMF (5 mL). The reaction was then allowed to stir at room temperature under argon. After 3 days, the solvent was removed by rotary-evaporation under vacuum. The resulting solid was then placed under argon and then dissolved in 4N HCl in Dioxane (2mL) and then allowed to stir at room temperature under a blanket of argon. After 1 hour, the dioxane and HCl were removed by rotary-evaporation under vacuum. The solid was then suspended in methanol (2 mL) and once more brought to dryness by rotary-evaporation in order to remove residual HCl and dioxane. This material was then resuspended in methanol (2 mL) and precipitated by the addition of water (20 mL). The aqueous suspension was then stored at 4°C for 4 hours, and the solid isolated by

centrifugation. The pelleted material was then allowed to dry under vacuum over night. This process afforded a mixture of $(Glu)_n$ and $(Glu)_n$-cephalexin (464mg) as determined by MALDI. MALDI indicates a mixture of polymers $(Glu)_{7\text{-}13}$ (SEQ ID NO: 1) and $(Glu)_{5\text{-}14}$-cephalexin (SEQ ID NO: 2). Other chain-lengths may be present but they are not clearly visible in the MALDI spectra. Reversed-phase HPLC (265nm detection, C18 column, 16%MeOH/4%THF/80%water mobile phase) indicated that no free cephalexin was present in the isolated material. "Water" in the HPLC actually refers to an aqueous buffer of 0.1% heptanesulfonic acid and 1.5% triethylamine.

II:C – Example: Synthesis of PolyGlu-Atenolol

[156] The following procedure was successfully used to synthesize the polyglutamic acid conjugate of atenolol.



MW ($g.mol^{-1}$)	266.3	173	2202 (n=15)
Mass (mg)	616	2000	1307 = 100%
mmoles	2.31	11.56	0.77
Equivalents	1	5	0.33

DMF is dimethylformamide, anhydrous, and was purchased from Aldrich. Glassware was oven-dried prior to use.

[157] Glu-NCA (2 g, 11.56 mmoles) was dissolved in 8 mL of DMF and stirred under Ar in a 25 mL roundbottom flask equipped with a gas inlet tube. Atenolol, dissolved in 2 mL of DMF, was added to this solution of Glu-NCA and allowed to stir at room temperature for 93 h. Bubbles were observed at the beginning of the reaction. The DMF was reduced by rotary evaporation and the oil was transferred into a 125 mL Erlenmeyer, rinsing the round bottom well with water. The pH of the solution was adjusted to 3 with 1 N HCl. This solution (60

mL total volume) was then cooled in the refrigerator for > 3 hours. The suspension was filtered through a sintered glass funnel and washed with 3 X 30 mL of 1% AcOH in methanol followed by 3 X 30 mL of ethanol. The solid was then dried by high vacuum overnight yielding 892 mg (68%) of polymer. NMR analysis indicated that the Glu/Atenolol ratio was about 15/1. This is based on the relative integrations of the methyl groups on the N-isopropyl substituent of atenolol (6 protons) and the β and γ protons of the Glu (2 each).

II:D – Example: Synthesis of $[Glu]_{15}$-Carbadopa (SEQ ID NO: 3)

[158] To 50 mg of Carbadopa (0.22 mmoles) dissolved in 4 mL of dry dimethylformamide, add 573 mg (3.3 mmoles) of GluNCA. Stir overnight under Argon. After the addition of 12 mL of H_2O, pH = 2.0, the solution was ultrafiltered (regenerated cellulose, Millipore, YM1, NMWL = 1000) with an additional 100 mL of H_2O, pH = 2. The resulting precipitate was collected by filtration and washed with 30 mL of H_2O and dried in a vacuum at room temperature to yield 268 mg of a light brown powder. The Carbadopa to Glu ratio was determined by ^1H NMR to be 1:4. ^1H NMR (500MHz, DMSO) peak assignments are as follows: δ6.61-6.58 (aromatic, Carbadopa), 6.47-6.41(aromatic, Carbadopa), 4.25 (α, Glu), 2.25 (γ, Glu), 2.00-1.65 (β, Glu), 1.11 (CH_3, Carbadopa).

II:E – Example: Preparation of Drug-Glu Conjugate as a starting synthon for polymerization

[159] The example below is a description C-terminus attachment. The example describes how to attach a single amino acid to an active agent, one of the preferred embodiments. The example also provides an active agent/amino acid conjugate to which additional amino acids can be added resulting in the desired peptide. Preferred embodiments of the peptide include copolymers of glutamic acid and N-acetylcysteine.

[160] With non-primary amine drug candidates, formation of the active agent-poly-Glu conjugate may require the formation of the activate agent/amino acid

synthon prior to polymerization. The following scheme was used, wherein the active agent is first conjugated to Glu, and this synthon is then used to initiate coupling. Examples of this protocol are further described as applied to sertraline, propranolol and metoprolol.

II:F – Protocol for coupling Boc-Glu(OtBu)-OH to Sertraline

Boc-Glu(OtBu)-OH (0.44 g, 1.46 mmol) and PyBOP (0.84 g, 1.60 mmol) were dissolved in dry DMF (15 mL) with stirring. DIEA (0.31 mL, 1.75 mmol) was added and the amino acid derivative was allowed to activate for 15 minutes. Sertraline hydrochloride (0.50 g, 1.46 mmol) was added to the stirring mixture followed by an additional 0.31 mL DIEA. The mixture was allowed to stir for 16 hours. The solution was stripped yielding brown oil. The oil was dissolved in EtOAc (100 mL) and the resulting solution was washed with 10 % HCl (3 x 30 mL), saturated $NaHCO_3$, 4M $NaHSO_4$, and brine (2 x 30 mL, respectively). The solution was dried over $MgSO_4$, filtered and the solvent was removed by rotary evaporation under reduced pressure, yielding light brown oil. The oil was dried on the vacuum manifold and the product was purified by column chromatography on silica gel using EtOAc/Hexanes 1:5 to 1:4 solvent system. The product fractions were pooled and solvent was again removed by rotary evaporation yielding 0.85 g (99%) of the final product, Sertraline-NH-C(O)-Glu-NH3+. The preparation was dried on the vacuum manifold.

II:G – Synthesis of PolyGlu-Propranolol

(i) **Protocol for coupling Boc-Glu(OtBu)-OH to Propranolol**

[161] Boc-Glu(OtBu)-OH (0.44 g, 1.46 mmol) and PyBOP (0.84 g, 1.60 mmol) were dissolved in dry DMF (15 mL) with stirring. DIEA (0.31 mL, 1.75 mmol) was added and the amino acid derivative was allowed to activate for 15 minutes. Propranolol hydrochloride (0.43 g, 1.46 mmol) was added to the stirring mixture followed by an additional 0.31 mL DIEA. The mixture was allowed to stir for 16 h. The solution was stripped yielding a brown oil. The oil was dissolved in EtOAc (100 mL) and the resulting solution was washed with 10 % HCl (3 x 30 mL),

saturated $NaHCO_3$, 4M $NaHSO_4$, and brine (2 x 30 mL, respectively). The solution was dried over $MgSO_4$, filtered and the solvent was removed by rotary evaporation under reduced pressure, yielding light brown oil. The oil was dried on the vacuum manifold and the product was purified by column chromatography on silica gel using EtOAc/Hexanes 1:5 to 1:4 solvent system. The product fractions were pooled and solvent was again removed by rotary evaporation the final product, Propranolol-NH-C(O)-Glu-NH3+. The preparation was dried on the vacuum manifold.

(ii) Protocol for Initiating Glu-NCA Polymerization with Propranolol-Glu

[162] The above synthon was used in a reaction similar to the one provided for atenolol.

II:H – Example: Synthesis of PolyGlu-Metroprolol

[163] This synthesis was identical to that described for PolyGlu-Propranolol.

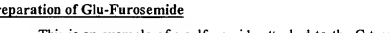
II:I – Example: Preparation of PolyGlu-Prednisone

[164] The example below describes the alcohol initiating polymerization of Glu-NCA to produce a C-terminus ester/drug conjugate.

[165] To GluNCA (0.128 g, 738 μmol) in 4 mL dry DMF was added Glu(21-Prednisone) (0.045 g, 92 μmol). After stirring for 68 h under Ar, 30 mL H_2O was added. The reaction was acidified to pH 4 with 1 N HCl and concentrated under vacuum. ^1H NMR (DMSO) analysis indicated 13:1 Glu:Prednisone ratio.

II:J - Preparation of Glu-Furosemide

[166] This is an example of a sulfonamide attached to the C-terminus of a peptide.

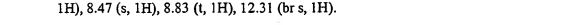


Reagents	MW	Weight	mmoles	Molar Equivalents
Furosemide	330.7	1.98g	1.2	1.2
Boc-Glu(OtBu)-OSu	400	2.00g	1.0	1.0
NMM	101	0.17ml	1.5	1.5
Dioxane	-	45ml	-	-

Glu-Furosemide

[167] To a solution of Furosemide in dioxane was added NMM followed by Boc-Glu(OtBu)-OSu. The solution was stirred at ambient temperatures for 18hours. Reaction was quenched with sat. NaHCO3 (25ml) and solvent was removed. Crude material was purified using preparative HPLC (Phenomenex Luna C18, 30X250mm, 5μM, 100Å; Gradient: 70 0.1%TFA-water/30 0.1% TFA-MeCN → 0/100 0-15min.; 30ml/min.). Solid was collected as a white powder (0.054g, 9% yield): ^{1}H NMR (DMSO-d_6) δ 1.40 (m, 18H), 1.53 (m, 1H), 1.62 (m, 1H), 1.85 (m, 2H), 3.95 (m, 1H), 4.60 (d, 2H), 6.42 (d, 2H), 7.00 (d, 1H), 7.07 (s, 1H), 7.62 (s, 1H), 8.47 (s, 1H), 8.83 (t, 1H), 12.31 (br s, 1H).

II: K - Preparation of Ala-Pro-Hydrocodone through the Enolate of Hydrocodone



Reagents	MW	Weight	mmoles	Molar Equivalents
Pro-Hydrocodone	468	0.25g	0.53	1.0
Boc-Ala-OSu	286	0.33g	1.2	2.26
NMM	101	0.50ml	5.38	10.2
DMF	-	10ml	-	-

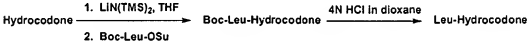
Ala-Pro-Hydrocodone

[168] To a solution of Pro-Hydrocodone in DMF was added NMM followed by Boc-Ala-OSu. The solution was stirred at ambient temperatures for 18hours. Solvent was removed. Crude material was purified using preparative HPLC (Phenomenex Luna C18, 30X250mm, 5μM, 100Å; Gradient: 100 water/0 0.1% TFA-MeCN → 0/100; 30ml/min.). Solid was collected as a slightly yellow

powder (0.307g, 85% yield): ^{1}H NMR (DMSO-d_6) δ 1.16 (d, 3H), 1.35 (s, 9H), 1.51 (m, 2H), 1.86-2.10 (m, 6H), 2.50 (m, 1H), 2.54 (m, 1H), 2.69 (m, 1H), 2.88 (s, 3H), 3.02 (dd, 1H), 3.26 (d, 1H), 3.55 (m, 1H), 3.67 (m, 1H), 3.72 (s, 3H), 3.80 (s, 1H), 4.25 (m, 1H), 4.43 (d, 1H), 5.01 (s, 1H), 5.59 (d, 1H), 6.75 (d, 1H), 6.88 (d, 1H), 6.99 (t, 1H), 9.91 (br s, 1H).

[169] To the Boc-Ala-Pro-Hydrocodone (0.100g) was added 10ml of 4N HCl in dioxane. The resulting mixture was stirred at ambient temperatures for 18 hours. Solvent was removed and final product dried under vacuum. Solid was collected as a slightly yellow solid (0.56g, 71% yield): ^{1}H NMR (DMSO-d_6) δ 1.38 (s, 3H), 1.48 (t, 1H), 1.80-2.29 (m, 8H), 2.65 (m, 1H), 2.80 (s, 3H), 2.96 (m, 3H), 3.23 (m, 2H), 3.76 (s, 3H), 3.92 (s,1H), 4.22 (s, 1H), 4.53 (s, 1H), 5.00 (s, 1H), 5.84 (d, 1H), 6.77 (d, 1H), 6.86 (d, 1H), 8.25 (br s, 3H).

II: L - Preparation of Leu-Hydrocodone



Reagents	MW	Weight	mmoles	Molar Equivalents
1. Hydrocodone	299	1.00g	3.34	1.0
1. LiN(TMS)$_2$ in THF	1M	10.5ml	10.5	3.15
1. THF	-	25ml	-	-
2. Boc-Leu-OSu	328	3.28g	10.0	3.0

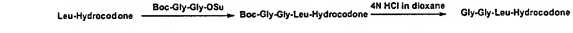
Leu-Hydrocodone

[170] To a solution of hydrocodone in THF was added LiN(TMS)$_2$ in THF via syringe. The solution was stirred at ambient temperatures for 5 minutes then Boc-Leu-OSu was added. The resulting reaction mixture was stirred at ambient temperatures for 18 hours. Reaction was neutralized to pH 7 with 6M HCl. Solvent was removed. Crude material was taken up in $CHCl_3$ (100ml), washed with sat.

$NaHCO_3$ (3X100ml), dried over $MgSO_4$, filtered, and solvent removed. Solid was collected as a yellow powder (1.98g, 95% yield): ^1H NMR (DMSO-d_6) δ 0.86 (dd, 6H), 1.31 (s, 9H), 1.46 (s, 2H), 1.55 (m, 2H), 1.69 (m, 1H), 1.87 (dt, 1H), 2.07 (dt, 2H), 2.29 (s, 3H), 2.43 (m, 2H), 2.93 (d, 1H), 3.11 (s, 1H), 3.72 (s, 3H), 3.88 (dt, 1H), 4.03 (dt, 1H), 4.87 (s, 1H), 5.51 (d, 1H), 6.65 (d, 1H), 6.73 (d, 1H), 6.90 (s, 1H).

[171] To the Boc-Leu-Hydrocodone was added 25ml of 4N HCl in dioxane. The resulting mixture was stirred at ambient temperatures for 18 hours. Solvent was removed and final product dried under vacuum. Solid was collected as a slightly yellow solid (1.96g, 97% yield): ^1H NMR (DMSO-d_6) δ 0.94 (d, 6H), 1.52 (m, 1H), 1.75-1.90 (m, 4H), 2.22 (dt, 1H), 2.34 (dt, 1H), 2.64 (q, 1H), 2.75 (s, 3H), 2.95-3.23 (m, 4H), 3.74 (s, 3H), 3.91 (d, 1H), 4.07 (s, 1H), 5.10 (s, 1H), 5.72 (d, 1H), 6.76 (d, 1H), 6.86 (d, 1H), 8.73 br s, 3H).

II: M - Preparation of Gly-Gly-Leu-Hydrocodone



Reagents	MW	Weight	mmoles	Molar Equivalents
Leu-Hydrocodone	484	2.21g	4.56	1.0
Boc-Gly-Gly-OSu	329	3.00g	9.12	2.0
NMM	101	5.0ml	45.6	10
DMF	-	100ml	-	-

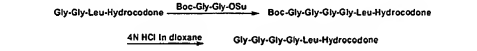
Gly-Gly-Leu-Hydrocodone

[172] To a solution of Leu-Hydrocodone in DMF was added NMM followed by Boc-Gly-Gly-OSu. The solution was stirred at ambient temperatures for 18hours. Solvent was removed. Crude material was purified using preparative HPLC (Phenomenex Luna C18, 30X250mm, 5μM, 100Å; Gradient: 90 water/10 0.1% TFA-MeCN → 0/100; 30ml/min.). Solid was collected as a slightly yellow powder (2.08g, 73% yield): ^1H NMR (DMSO-d_6) δ 0.88 (dd, 6H), 1.38 (s, 9H),

1.53-1.72 (m, 5H), 1.89 (d, 1H), 2.15 (m, 1H), 2.67 (m, 2H), 2.94 (s, 3H), 3.05 (m, 2H), 3.25 (m, 2H), 3.56 (d, 3H), 3.76 (s, 6H), 3.98 (s, 1H), 4.35 (q, 1H), 5.04 (s, 1H), 5.59 (d, 1H), 6.77 (d, 1H), 6.85 (d, 1H), 7.04 (t, 1H), 8.01 (t, 1H), 8.30 (d, 1H), 9.99 (br s, 1H).

[173] To the Boc-Gly-Gly-Leu-Hydrocodone (2.08g) was added 50ml of 4N HCl in dioxane. The resulting mixture was stirred at ambient temperatures for 18 hours. Solvent was removed and final product dried under vacuum. Solid was collected as a slightly yellow solid (1.72g, 86% yield): ^{1}H NMR (DMSO-d_6) δ 0.89 (dd, 6H), 1.50-1.87 (m, 5H), 2.26 (m, 2H), 2.66 (m, 2H), 2.82-2.97 (m, 5H), 3.21 (m, 2H), 3.60 (m, 4H), 3.88 (m, 5H), 4.37 (m, 1H), 5.04 (s, 1H), 5.60 (s, 1H), 6.79 (d, 2H), 8.07 (br s, 3H), 8.54 (br s, 1H), 8.66 (br s, 1H), 11.29 (br s, 1H).

II: N - Preparation of Gly-Gly-Gly-Gly-Leu-Hydrocodone



Reagents	MW	Weight	mmoles	Molar Equivalents
Gly-Gly-Leu-Hydrocodone	599	0.580g	0.970	1.0
Boc-Gly-Gly-OSu	329	0.638g	1.94	2.0
NMM	101	1.06ml	9.70	10
DMF	-	20ml	-	-

Gly-Gly-Gly-Gly-Leu-Hydrocodone

[174] To a solution of Gly-Gly-Leu-Hydrocodone in DMF was added NMM followed by Boc-Gly-Gly-OSu. The solution was stirred at ambient temperatures for 18hours. Solvent was removed. Crude material was purified using preparative HPLC (Phenomenex Luna C18, 30X250mm, 5μM, 100Å; Gradient: 85 water/15 0.1% TFA-MeCN → 50/50; 30ml/min.). Solid was collected as a slightly yellow powder (0.304g, 37% yield).

[175] To the Boc-Gly-Gly-Gly-Gly-Leu-Hydrocodone (0.304g) was added 25ml of 4N HCl in dioxane. The resulting mixture was stirred at ambient

temperatures for 18 hours. Solvent was removed and final product dried under vacuum. Solid was collected as a slightly yellow solid (0.247g, 97% yield): 1H NMR (DMSO-d6) δ 0.87 (m, 6H), 1.23 (s, 1H), 1.51-1.86 (m, 4H), 2.18 (m, 1H), 2.71 (m, 2H), 2.77 (s, 3H), 2.96 (m, 2H), 3.17 (m, 2H), 3.61 (s, 3H), 3.81-3.84 (m, 10H), 4.22 (m, 1H), 4.36 (m, 1H), 5.09 (m, 1H), 5.59 (d, 1H), 6.74 (dd, 2H), 8.16 (br s, 4H), 8.38 (br s, 1H), 8.74 (br s, 1H), 11.42 (br s, 1H).

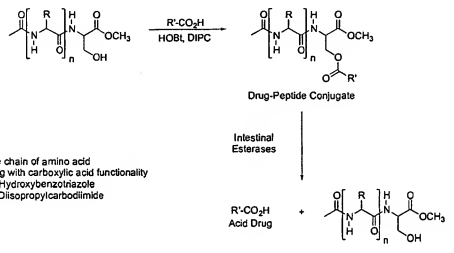
III Side-chain attachment of drugs to a Peptide

[176] The attachment of active agents to the side-chain of a peptide can be formed through a plurality of a combination of functional groups that can be selected from the active agent or amino acid used for conjugation. Unlike, when the active agent is conjugated to the N-terminus or C-terminus, where the functional group of the amino acid is restricted to either an amine or carboxylate group respectively, side-chain attachment allows for variability in the selection of specific amino acid side-chain functionalities. Additionally, where applicable the active agent functional group can be selected to conform to the amino acid side-chain utilized for attachment. The functional groups depend on the functionality on the side chain of a peptide utilized for conjugation. The diversity of side-chain attachment allows any active agent to be directly attached to the side chain of amino acids with appropriate functional groups. Active agents containing alcohols, amines and/or carboxylic acids are directly amenable to attachment through and may dictate the side-chain of the amino acid selected. For active agents that lack these functional groups it is preferred that the incorporation of a linker contain an alcohol, amine, or carboxylic group.

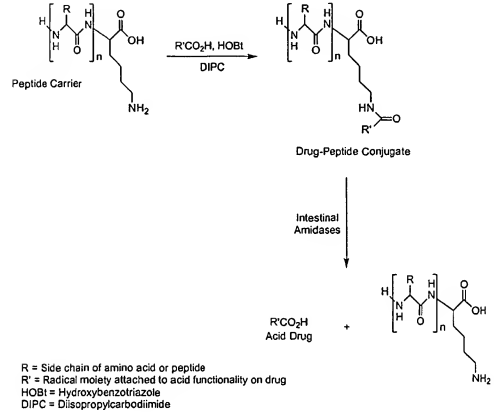
[177] More preferred amino acids used to create the attachment and/or the peptide are glutamic acid, aspartic acid, serine, lysine, cysteine, threonine, and glutamine. While homopolymers are often used, heteropolymers may be utilized to impart specific performance parameters. These heteropolymers may be of various chain length and degree of heterogeneity. Preferred examples include, but are not

limited to, dipetides including Leu-Ser, Leu-Glu, homopolyers of Glu and Leu and heteropolymers of (Glu)n-Leu-Ser.

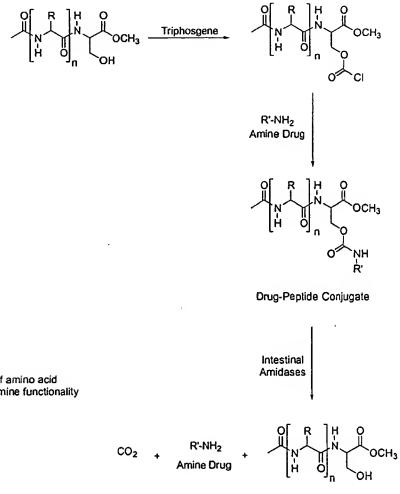
[178] An example of side-chain attachment conjugation to an acid drug is depicted in the scheme below:



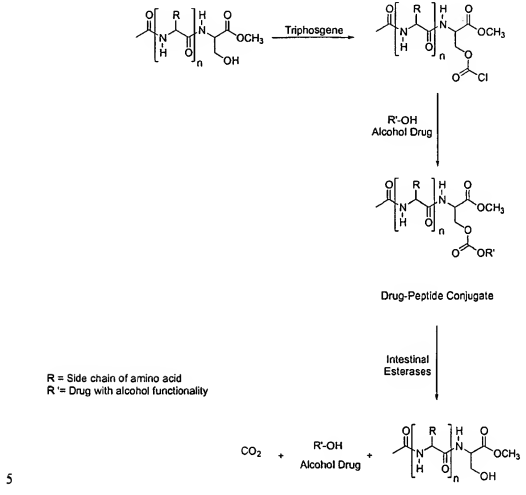
[179] The below example is a depiction of a side-chain attachment of an acid drug. In this case the amino acid depicted is lysine:



[180] An example of side-chain attachment conjugation to an amine drug is depicted in the scheme below:



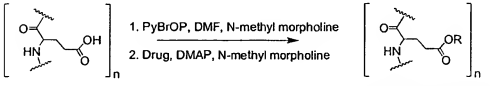
[181] An example of side-chain attachment conjugation to an alcohol drug is depicted in the scheme below:



[182] The below examples describe the general procedure for attachment of an amino acid active agent to the side-chain of a peptide. Examples III:A - III:G describe the attachment of active agents to a peptide through the alcohol group. One of the examples describes the attachment of Naltrexone to aspartic acid, while the others show different active agents attached to glutamic acid. Examples III:H - III:I are illustrative of the conversion of one of the naturally occurring amino acids, in this case Glu, to a glutamic acid derivative for subsequent incorporation into a peptide either through the NCA method or through the use of a peptide synthesizer. Example III:J is further illustrative of the conversion of one of the naturally occurring amino acids, in this case Glu, to a glutamic acid derivative which can be further incorporated into a linear or dendritic peptide either through the NCA method or through the use of a peptide synthesizer. Examples III:K - III:N show a carboxylic acid attached to the side chain of an amino acid. In the present examples, the active agent is attached to Polylysine through the amino group. Example III:O is describes a sulfonamide attached to the side-chain of a Polyglutamic acid.

[183] Specific examples of active agent attached to the side-chain attachment below are meant for example purposes only and are not meant to limit the invention to either specific active agents, amino acids or combinations thereof. Those skilled in the art would recognize from the present disclosure other active agents, which can be attached to the side-chain of a peptide.

III:A - Attachment of an Active Agent via an Alcohol Group to the Sidechain of Peptide

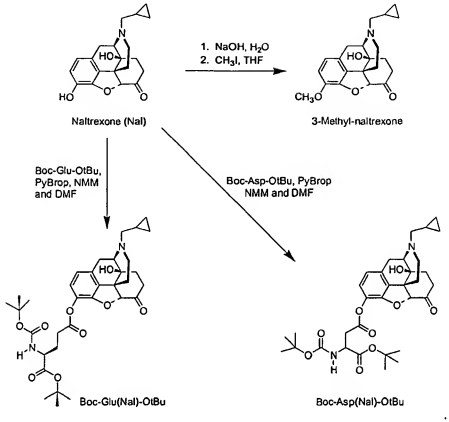


R = Drug attached via Alcohol Group as an Ester

Reagents	MW	Molar Equivalents
1. poly(glutamic acid)	128 per residue	1 per residue
1. PyBrOP	466	0.2-2.0
1. N-methyl morpholine	101	2.1-3.1
1. DMF	-	-
2. Drug with alcohol	-	0.3-2.0
2. N-methyl morpholine	101	1.3-3.1
2. DMAP	122	0.1-2.0

[184] To a solution of poly(glutamic acid) in DMF was added N-methyl morpholine and bromo-*tris*-pyrrolidino-phosphonium hexafluorophosphate (PyBrOP). The resulting mixture was allowed to stir at room temperature for 30-60 minutes. After this time, N-methyl morpholine and DMAP followed by drug were added. The resulting solution was stirred at room temperature or at 60° C for 24 to 48 hours. Solvent and excess base were then removed using reduced pressure evaporation. Water, methanol or *i*-propanol was then added and the resulting solid was collected and dissolved in $NaHCO_3$(sat.). The crude product was purified using ultrafiltration. Product was then collected from ultrafiltration using acid precipitation, methanol precipitation, acetone precipitation or removal of water under reduced pressure.

III:B – Example: Naltrexone Derivatives



(i) **Boc-Glu(Nal)-OtBu:**

[185] The following examples describe the attachment of different alcohol active agents to the side chain of glutamic acid to produce a new ester bond.

[186] The solids Boc-Glu-OtBu (0.96 g, 3.18 mmol), naltrexone (1.00 g, 2.65 mmol) and PyBrop (1.73 g, 3.71 mmol) were dissolved in 5 mL of anhydrous DMF and stirred at room temperature under argon. Dry N-methylmorpholine (1.08 mL, 9.81 mmol) was added and the reaction allowed to continue stirring at room temperature under argon. After two days additional Boc-Glu-OtBu (0.096 g, 0.32 mmol), PyBrop (0.173 g, 0.37 mmol) and N-methylmorpholine (0.10 mL, 0.981 mmol) were added. After 2 more days, the solvent was removed by rotary-evaporation under high vacuum. The resulting residue was then dissolved in $CHCl_3$,

and the resulting organic solution extracted with 2 x 20 mL of saturated NaCl, 3 x 20 mL of 10% Na_2CO_3 and a final wash with 20 mL of saturated aqueous NaCl. The organic solution was collected, dried over sodium sulfate and then adsorbed onto silica. Pure naltrexone conjugated amino acid (0.486 g, 0.78 mmol, 29%) was then isolated by flash chromatography and a gradient of 0-1.5% CH_3OH in $CHCl_3$. The purity of the isolated material was determined by TLC (6:1 CH_3OH:$CHCl_3$), and 1H NMR confirmed the presence of both the amino acid moiety and the naltrexone.

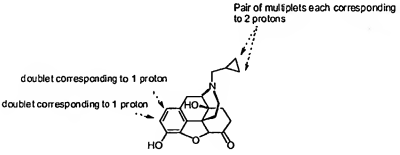
^{1}H NMR (360 MHz, $CDCl_3$): δ 6.81 (d, 1H, naltrexone aromatic), 6.63 (d, 1H, naltrexone aromatic), 4.3-4.2 (m, 1H, glutamic acid α-proton), 1.7-1.3 (pair of bs, 18H, Boc and OtBu groups.), 0.6-0.4 ppm (m, 2H, naltrexone cyclopropyl) and 0.2-0.0 ppm (m, 2H, naltrexone cyclopropyl).

(ii) **Boc-Asp(Nal)-OtBu**

[187] Boc-Asp(Nal)-OtBu was obtained in 41% isolate yield using a similar protocol as the one used to prepare Boc-Glu(Nal)-OtBu.

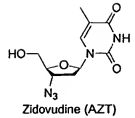
^{1}H-NMR (360 MHz, $CDCl_3$): δ 6.84 (d, 1H, naltrexone aromatic), 6.66 (d, 1H, naltrexone aromatic), 4.6-4.5 (m, 1H, aspartic acid α-proton), 1.6-1.3 (pair of bs, 18H, Boc and OtBu groups.), 0.7-0.5 ppm (m, 2H, naltrexone cyclopropyl) and 0.4-0.1 ppm (m, 2H, naltrexone cyclopropyl).

NMR characterization:



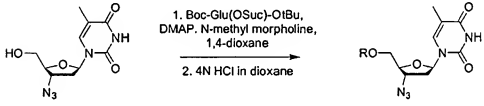
[188] While naltrexone has a complex NMR spectrum, there are several key protons that have distinct chemical shifts and are unique to naltrexone.

III:C – Example: Glu(AZT)



Zidovudine (AZT)

1-(4-Azido-5-hydroxymethyl-tetrahydro-furan-2-yl)-5-methyl-1*H*-pyrimidine-2,4-dione



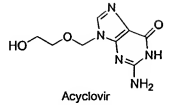
[189] To a solution of zidovudine (1.00 g, 3.75 mmol) and Boc-Glu(OSuc)-OtBu (3.00 g, 7.49 mmol) in dioxane (75 mL) was added DMAP (0.137 g, 1.13 mmol) and N-methyl morpholine (0.82 mL, 7.49 mmol). The solution was heated to reflux for 6 hours and heated at 70°C for 12 hours. Solvent was then removed and the crude product was purified over silica gel (100% $CHCl_3$) to obtain Boc-Glu(AZT)-OtBu (1.09 g, 1.91 mmol, 51%) as a yellow foam.

^{1}H NMR (360 MHz, $CDCl_3$): δ 1.40 (d, 32H, t-Bu), 1.86 (s, 3H, AZT CH_3), 2.11 (m, 2H, Glu-β H), 2.38 (m, 4H, Glu-γ H and AZT 2' CH_2), 4.00-4.31 (m, 4H, AZT 4' CH, 5' CH_2 and Glu-αH), 5.21 (d, 1H, AZT 3' CH), 6.01 (t, 1H, AZT 1' CH), 7.16 (s, 1H, AZT 6 CH).

[190] A solution of Boc-Glu(AZT)-OtBu (1.09 g, 1.91 mmol) in 4N HCl in dioxane (20 mL) was stirred for 4 hours and solvent removed. The product, Glu(AZT) (0.89 g, 1.99 mmol, quant.), was obtained as a yellow glass.

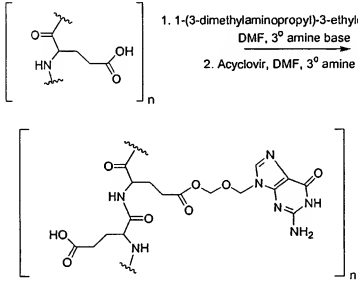
^{1}H NMR (360 MHz, D_2O): δ 1.89 (s. 3H, AZT CH_3), 2.21 (m, 4H, Glu-β H and AZT 2' CH_2), 2.58 (m, 2H, Glu-γ H), 3.70 (t, 1H, Glu-α H), 4.05-4.41 (m, 4H, AZT 4' CH, 3' CH and 5' CH_2), 6.18 (t, 1H, AZT 1' CH), 7.51 (s, 1H, AZT 6 CH).

III:D – Example: Poly-Glu(Acyclovir)



Acyclovir

2-Amino-9-(2-hydroxy-ethoxymethyl)-1,9-dihydro-purin-6-one

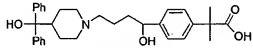


[191] To a solution of poly-glu_{15} (SEQ ID NO: 3) (0.600 g, 0.310 mmol) in DMF (25 mL) was added EDCI (2.07 g, 10.8 mmol). The resulting mixture was allowed to stir at ambient temperature for one hour. Then, N-methyl morpholine (0.51 mL, 4.7 mmol) was added followed by a mixture of acyclovir (1.74 g, 7.75 mmol), DMF (25 mL) and N-methyl morpholine (0.85 mL). The reaction mixture was stirred at ambient temperature for 4 days. After this, water (50 mL) was added and all solvent was removed. To the dried mixture was added water (100 mL) and a precipitate of unreacted acyclovir formed. Solid was centrifuged and the supernatant was purified using ultrafiltration (YM1 membrane). Approximately 300 mL water was allowed to pass through the membrane. NMR has shown an

unexpected alkyl-urea side chain attached impurity. Poly-glu(acyclovir) (0.97 0g) was obtained as a light yellow solid.

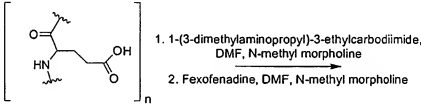
^{1}H NMR (360 MHz, D_2O): δ 1.11 (br m, 4H, urea), 2.01 (br m, 2H, Glu-β H), 2.39 (br m, 2H, Glu-γ H), 2.72 (br m, 2H, urea), 3.32 (br m, 6H, acyclovir CH_2 and urea), 3.83 (br m, 3H, urea), 4.38 (br d, 3H, Glu-α H), 5.47 (br s, 2H, acyclovir 1' CH2), 7.94 (br s, 1H, acyclovir 8 CH).

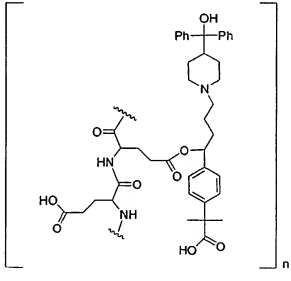
III:E – Example: Poly-Glu(Fexofenadine)



Fexofenadine

2-(4-{1-Hydroxy-4-[4-(hydroxy-diphenyl-methyl)-piperidin-1-yl]-butyl}-phenyl)-2-methyl-propionic acid



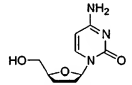


[192] To a solution of poly-glu_{15} (SEQ ID NO: 3)(0.078 g, 0.040 mmol) in DMF (5 mL) was added EDCI (0.035 g, 0.18 mmol). After stirring for 30 minutes,

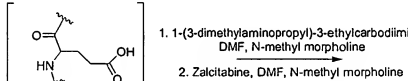
N-methyl morpholine was added (0.03 mL, 0.24 mmol). After stirring for 10 minutes, a solution of fexofenadine (0.100 g, 0.20 mmol), N-methyl morpholine (0.07 mL, 0.60 mmol) and DMF (5 mL) was added via a syringe. After stirring reaction at ambient temperatures for three days, sample was dissolved in water (25 mL). A solid precipitate formed which was both drug-conjugate and free fexofenadine. Water was acidified and all solids dissolved. Purification using ultrafiltration (YM1 followed by YM3) and size exclusion chromatography using Sephadex-25 at pH 7 yielded poly-glu(fexofenadine) (0.010 g) as a white solid.

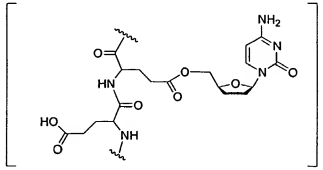
^{1}H NMR (360 MHz, D_2O): δ 1.37 (s, 8H, fex. CH_2 and CH_3), 1.58 (br m, 5H, fex. CH and CH_2), 1.99 (br m, 24H, Glu-β H), 2.31 (br m, 24H, Glu-γ H), 2.70 (br m, 10H, fex. CH and CH_2), 4.14 (br m, 26H, Glu-α H), 7.25 (br m, 14H, fex. aromatic H).

III:F – Example: Poly-Glu(Zalcitabine)



Zalcitabine
4-Amino-1-(5-hydroxymethyl-tetrahydro-furan-2-yl)-1*H*-pyrimidin-2-one

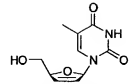




[193] To a solution of poly-glu_{15} (SEQ ID NO: 3) (0.123 g, 0.060 mmol) in DMF (8 mL) was added EDCI (0.403 g, 2.10 mmol). After 30 minutes, N-methyl morpholine (0.13 mL, 1.2 mmol) was added. After 35 minutes, a solution of zalcitabine (0.200 g, 0.95 mmol), N-methyl morpholine (0.10 mL, 0.9 mmol) and DMF (2 mL) was added via a syringe. The resulting mixture was stirred at ambient temperature for 48 hours. Solvent was removed and the residue was dissolved in water (15 mL). Ultrafiltration (YM1 followed with YM3) and size exclusion using Sephadex-25 at pH 7 yielded poly-glu(zalcitabine) (0.083 g) as a light yellow solid.

^{1}H NMR (360 MHz, DMSO-d_6 w/D_2O): δ 1.14 (br m, 20H, urea), 1.90 (br m, 30H, Glu-β H, Glu-γ H and CH_2 in zalcitabine), 2.66 (br m, 4H, urea), 3.24 (br m, 36H, urea, CH and CH_2 in zalcitabine), 4.29 (br m, 8H, Glu-α H), 5.87 (br s, 1H, zalcitabine 1' CH), 7.18 (br s, 1.19H, zalcitabine NH_2), 8.52 (br s, 1H, zalcitabine 6 CH).

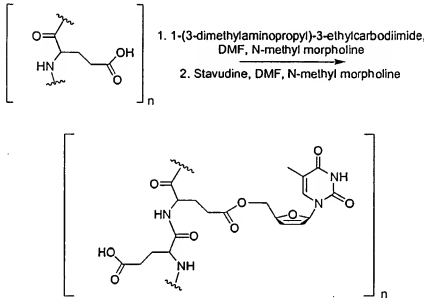
III:G – Example: Poly-Glu(Stavudine)



Stavudine

1-(5-Hydroxymethyl-2,5-dihydro-furan-2-yl)-5-methyl-1*H*-pyrimidine-2,4-dione

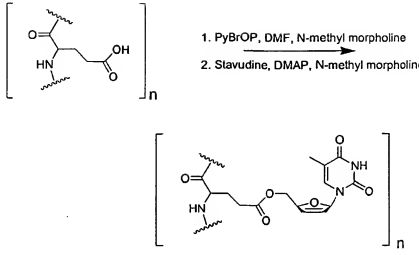
(i) **Method A**



[194] Preparation was similar to poly-Glu(zalcitabine). Purification using ultrafiltration (YM1) yielded poly-Glu(stavudine) (0.089 g) as a white solid.

^{1}H NMR (360 MHz, D_2O): δ 1.87 (s, 3H, stavudine 5 CH_3), 2.06 (br m, 38H, Glu-β H and Glu-γ H), 2.49 (br m, 12H, Glu-γ H), 3.75 (br m, 12H, urea and stavudine 5' CH_2), 3.96 (br m, 12H, urea), 4.45 (br d, 13H, Glu-α H), 5.98 (d, 1H, stavudine 1' CH), 6.48 (d, 1H, stavudine 3' CH), 6.96 (d, 1H, stavudine 2' CH), 7.63 (s, 1H, stavudine 6 CH).

(ii) **Method B**



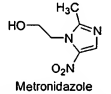
Reagents	Weight	MW	Molar Equivalents
1. poly(glutamic acid)	1.00 g	128 per residue	1 per residue
1. PyBrOP	2.91 g	466	0.8
1. N-methyl morpholine	1.80 mL	101	2.1
1. DMF	50 mL	-	-
2. Stavudine	1.57 g	224	0.9
2. N-methyl morpholine	1.11 mL	101	1.3
2. DMAP	0.191 g	122	0.2

[195] To a solution of poly(glutamic acid) (1.00 g, 7.81 mmol) in DMF (50 mL) was added N-methyl morpholine (1.80 mL, 16.4 mmol) and bromo-*tris*-pyrrolidino-phosphonium hexafluorophosphate (PyBrOP) (2.91 g, 6.25 mmol). The resulting mixture was allowed to stir at room temperature for 30 minutes. After this time, N-methyl morpholine (1.11 mL, 10.2 mmol) and 4-dimethylaminopyridine (0.191 g, 1.56 mmol) (DMAP) followed by Stavudine (1.57 g, 7.03 mmol) were added. The resulting solution was stirred at room temperature for 24 hours. Solvent and excess base were then removed using reduced pressure evaporation. Water was then added and the resulting solid was collected and dissolved in saturated $NaHCO_3$. The crude product was purified using ultrafiltration. Product was then collected from ultrafiltration using acid precipitation (1.15 g, 48%).

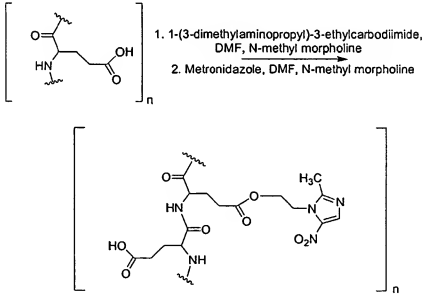
^{1}H NMR (360 MHz, DMSO-d_6): δ 1.73 (br s, 3H, stavudine 5 CH_3), 1.89 (br s, 4H, Glu-β H), 2.27 (br s, 4H, Glu-γ H), 4.16 (br m, 4H, Glu-α H and stavudine 5′ CH_2), 4.95 (br s, 1H, stavudine 4′CH), 5.97 (br s, 1H, stavudine 1′CH), 6.42 (br s, 1H, stavudine 3′CH), 6.80 (br s, 1H, stavudine 2′CH), 7.20 (br s, 1H, stavudine 6 CH), 8.06 (br s, 2H, Glu- NH), 11.37 (br s, 1H, stavudine NH), 12.14 (br s, 1H, Glu OH).

Stavudine UV λ_{max} (266nm), poly-glu(Stavudine) UV λ_{max} (266nm), Average % mass of Stavudine in poly-glu(Stavudine) 36%; MALDI: Glu_n(Stavudine) + NA n=6-8, $Glu_n(Stavudine)_2$ + NA n=4-7, $Glu_n(Stavudine)_3$ + NA n=2-8, $Glu_n(Stavudine)_4$ + NA n=3-10, $Glu_n(Stavudine)_5$ + NA n=5-13, $Glu_n(Stavudine)_6$ + NA n=7-14, Glu_n(Stavudine)7 + NA n=9-14.

III:H – Example: Poly-Glu(Metronidazole)



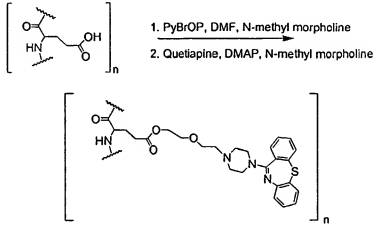
Metronidazole
2-(2-Methyl-5-nitro-imidazol-1-yl)-ethanol



[196] Preparation was similar to poly-glu(zalcitabine). Purification using ultrafiltration (YM1) yielded poly-Glu(metronidazole) (0.326 g) as a yellow solid.

1H NMR (360 MHz, DMSO-d_6): δ 1.18 (br d, 13H, urea), 1.93 (br s, 17H, Glu-β H and Glu-γ H), 2.71 (br s, 16H, urea), 4.01 (br m, 18H, Glu-α H and metronidazole CH_2), 4.58 (br s, 2H, metronidazole CH_2), 8.05 (br s, 1H, metronidazole 2 CH).

III:I – Example: Attachment of Quetiapine via Alcohol to the Sidechain of Poly(Glutamic Acid)



Reagents	Weight	MW	Molar Equivalents
1. poly(glutamic acid)	1.00 g	128 per residue	1 per residue
1. PyBrOP	2.55 g	466	0.7
1. N-methyl morpholine	1.80 mL	101	2.1
1. DMF	50 mL	-	-
2. Quetiapine	1.79 g	224	0.6
2. N-methyl morpholine	1.11 mL	101	1.3
2. DMAP	0.191 g	122	0.2

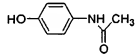
[197] To a solution of poly(glutamic acid) (1.00 g, 7.81 mmol) in DMF (50 mL) was added N-methyl morpholine (1.80 mL, 16.4 mmol) and bromo-*tris*-pyrrolidino-phosphonium hexafluorophosphate (PyBrOP) (2.55 g, 5.47 mmol). The resulting mixture was allowed to stir at room temperature for 30 minutes. After this time, N-methyl morpholine (1.11 mL, 10.2 mmol) and 4-dimethylaminopyridine (0.191 g, 1.56 mmol) (DMAP) followed by Quetiapine (1.79 g, 4.69 mmol) were added. The resulting solution was stirred at room temperature for 24 hours. Solvent and excess base were then removed using reduced pressure evaporation. Water was then added and the resulting solid was collected and dissolved in saturated $NaHCO_3$.

The crude product was purified using ultrafiltration. Product was then collected from ultrafiltration using acid precipitation (0.965 g, 35%).

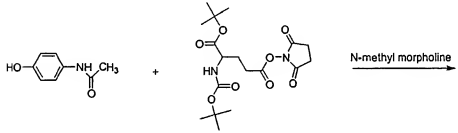
^{1}H NMR (360 MHz, DMSO-d_6): δ 1.87 (br d, 12H, Glu-β H), 2.33 (br m, 12H, Glu-γ H), 2.78 (br m, 8H, quetiapine), 3.49 (br m, 6H, quetiapine), 4.13 (br s, 2H, quetiapine), 4.22 (br s, 6H, quetiapine), 6.91 (br s, 1H, quetiapine), 7.01 (br s, 1H, quetiapine), 7.19 (br s, 1H, quetiapine), 7.38 (br m, 4H, quetiapine), 7.54 (br s, 1H, quetiapine), 8.07 (br s, 4H, Glu NH).

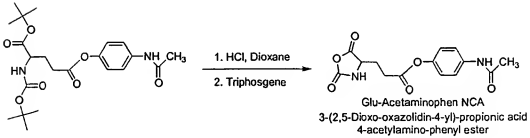
Quetiapine UV λ_{max} (250nm), poly-glu(Quetiapine) UV λ_{max} (250nm), Average % mass of Quetiapine in poly-glu(Quetiapine) 43%.

III:J – Example: 2-Amino-pentanedioic acid 5-(4-acetylamino-phenyl) ester or Glu(Acetaminophen)



Acetaminophen
N-(4-Hydroxy-phenyl)-acetamide





Glu-Acetaminophen NCA
3-(2,5-Dioxo-oxazolidin-4-yl)-propionic acid 4-acetylamino-phenyl ester

(i) **Preparation of Boc-Glu(Acetaminophen)-OtBu**

[198] To a solution of Boc-Glu(OSuc)-OtBu (0.500 g, 1.25 mmol) and acetaminophen (0.944 g, 6.25 mmol) in THF (15 mL) was added N-methyl morpholine (1.40 mL, 12.5 mmol). The reaction was allowed to heat to reflux and

stirred at reflux overnight. Solvent was then removed and the crude compound was purified over silica gel (50-75% ethyl acetate in hexanes) to obtain Boc-Glu(Acetaminophen)-OtBu (0.432 g, 0.900 mmol, 72%).

^{1}H NMR (360 MHz, $CDCl_3$): δ 1.43 (d, 18H, t-Bu), 1.97 (m, 1H, Glu-β H), 2.12 (s, 3H, acetaminophen CH_3), 2.25 (m, 1H, Glu-β H), 2.60 (m, 2H, Glu-γ H), 4.25 (m, 1H, Glu-α H), 7.04 (d, 2H, acetaminophen aromatic), 7.48 (d, 2H, acetaminophen aromatic).

(ii) Preparation of Glu(acetaminophen)

[199] A solution of Boc-Glu(Acetaminophen)-OtBu (0.097 g, 0.20 mmol) in 4N HCl in dioxane (10 mL) was stirred at ambient temperatures for 2 hours. Solvent was removed to obtain Glu(acetaminophen) (0.90 g) as the HCl salt.

^{1}H NMR (360 MHz, D_2O): δ 2.19 (s, 3H, acetaminophen CH_3), 2.41 (m, 2H, Glu-β H), 2.97 (t, 2H, Glu-γ H), 4.18 (t, 1H, Glu-α H), 7.19 (d, 2H, acetaminophen aromatic), 7.51 (d, 2H, acetaminophen aromatic).

^{13}C NMR (360 MHz, DMSO-d_6): δ 23.80, 29.25, 51.00, 66.24, 119.68, 121.69, 137.00, 145.35, 168.23, 170.42, 170.79.

(iii) Preparation of Glu(Acetaminophen) NCA

[200] To a mixture of 2-amino-pentanedioic acid 5-(4-acetylamino-phenyl) ester (1.54 g, 4.29 mmol) in THF (40 mL) was added triphosgene (1.02 g, 3.43 mmol). The resulting solution was stirred at reflux for 3 hours. During reaction, the product precipitated and was filtered away to obtain the NCA of Glu(acetaminophen) (1.02 g, 2.64 mmol, 62%) as an off white solid.

^{1}H NMR (360 MHz, DMSO-d_6): δ 2.01 (s, 3H, acetaminophen CH_3), 2.15 (m, 2H, Glu-β H), 2.81 (m, 2H, Glu-γ H), 3.76 (t, 1H, Glu-α H), 7.06 (d, 2H, acetaminophen aromatic), 7.63 (d, 2H, acetaminophen aromatic), 8.57 (br s, 1H, amide), 10.19 (s, 1H, amide).

^{13}C NMR (360 MHz, DMSO-d_6): δ 23.81, 29.25, 52.13, 54.62, 119.66, 121.71, 136.98, 145.35, 167.44, 168.19, 170.46, 170.77.

III:K – Example: Preparation of PolyGlu Prednisone

(i) BocGlu(21-Prednisone)O-tBu

[201] To BocGlu-O-tBu (0.400 g, 1.32 mmol) in 20 mL $CHCl_3$ was added dicyclohexylcarbodiimide (0.544 g, 2.64 mmol). The reaction was stirred for 1 hour and filtered to remove insoluble dicyclohexylurea. N-dimethyl-4-aminopyridine (0.320 g, 2.64 mmol) and prednisone (0.472 g, 1.32 mmol) was added. The reaction was stirred for 60 hours and filtered. The solvent was removed by rotary evaporation and the residue purified by flash chromatography (10:1-0:1 hexane:EtOAc) to provide the target as a clear film (0.256 g, 31%).

R_f = 0.54 (6:1 $CHCl_3$:MeOH; ^1H NMR (500 MHz, $CDCl_3$): δ 7.68 (d, 1H, 1), 6.16 (d, 1H, 2), 6.04 (s, 1H, 4), 5.15 (d, 1H, NH), 5.03 (d, 1H, 21), 4.71 (d, 1H, 21), 4.08 (t, 1H, α), 1.40 (s, 18H, t-Bu).

(ii) **Glu(21-Prednisone)**

[202] To BocGlu(21-Prednisone)O-tBu (0.060 g, 93 μmol) in 15 mL CH_2Cl_2 was stirred for 1 hour with trifluoroacetic acid (1.5 mL). The solvent was removed by rotary evaporation and the residue purified by flash chromatography (8:1 $CHCl_3$:MeOH) to yield a clear film.

R_f = 0.13 (6:1 $CHCl_3$:MeOH).

^1H NMR (500 MHz, $CDCl_3$): δ 7.72 (d, 1H, 1), 6.25 (d, 1H, 2), 6.14 (s, 1H, 4), 5.14 (d, 1H, 21), 4.75 (d, 1H, 21), 4.10 (t, 1H, α).

(iii) **Glu(21-Prednisone)NCA**

[203] To Glu(21-Prednisone) (0.044 g, 90 μmol) in 20 mL dry THF was added triphosgene (0.021 g, 72 μmol). After gently refluxing for 3 h, the solvent was removed by rotary evaporation, the residue washed thrice with 15 mL hexane and then dried under vacuum to yield the NCA as a white solid.

R_f = 0.98 (EtOAc).

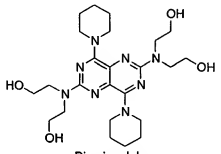
^1H NMR (500 MHz, $CDCl_3$): δ 7.72 (1H), 6.89 (1H), 6.25 (1H), 6.14 (s, 1H, 4), 5.14 (d, 1H, 21), 4.75 (d, 1H, 21), 4.49 (1H, α).

(iv) **PolyGlu(21-Prednisone)**

[204] Glu(21-Prednisone)NCA (0.037 g, 72 μmol) and Glu(21-Prednisone) (0.004 g, 8 μmol) were dissolved in 5 mL dry DMF. After stirring for 88 hours under argon the reaction mixture was poured into 30 mL H_2O and extracted thrice

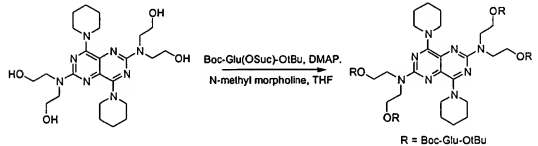
with 15 mL $CHCl_3$. The organic layer was concentrated and dried in vacuum to provide a drug bearing polymer with a 1:1 Prednisone:Glutamic acid ratio.

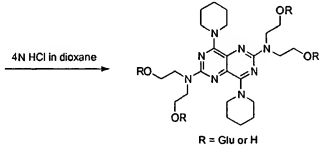
III:L – Example: Glu(Dipyrimadole)



Dipyrimadole

2-{(6-[Bis-(2-hydroxy-ethyl)-amino]-4,8-di-piperidin-1-yl-pyrimido[5,4-*d*]pyrimidin-2-yl} -(2-hydroxy-ethyl)-amino]-ethanol





(i) **Preparation of Boc-Glu(dipyrimadole)-OtBu**

[205] To a solution of dipyrimadole (0.500 g, 0.990 mmol) and Boc-Glu(OSuc)-OtBu (3.96 g, 9.91 mmol) in THF (35 mL) was added DMAP (0.072 g, 0.60 mmol) and N-methyl morpholine (0.22 mL, 1.98 mmol). The solution was then

refluxed for 48 hours. Solvent was then removed and crude product was purified over silica gel (25-50% ethyl acetate in hexanes). Two major products were isolated, one with R_f = 2-3, Boc-Glu(dipyrimadole)-OtBu, (0.57 g) and another with R_f = 3-4 (2.80 g), as bright yellow oils.

R_f = 2-3, 1H NMR (360 MHz, $CDCl_3$): δ 1.41 (s, 42H, t-Bu), 1.64 (br s, 5H, dipyrimadole), 1.85 (m, 2H, Glu-β H), 2.07 (m, 2H, Glu-β H), 2.37 (m, 4H, Glu-γ H), 3.60-4.24 (m, 12H, Glu-α H and dipyrimadole).

(for R_f = 3-4 similar as above except δ 1.44 (s, 56H, t-Bu)).

(ii) Preparation of Glu(dipyrimadole)

[206] A solution of Boc-Glu(dipyrimadole)-OtBu (R_f = 2-3, 0.57 g) and 4N HCl in dioxane (20 mL) was stirred at ambient temperature for 2.5 hours. Solvent was removed and the product (0.280 g) was a bright yellow solid.

^{1}H NMR (360 MHz, DMSO-d_6): δ 1.65 (br m, 4H, Glu-β H and dipyrimadole), 2.04 (br m, 2H, Glu-β H), 2.40 (br m, 4H, Glu-γ H), 3.75 (br m, 8H, dipyrimadole), 3.91 (br m, 2H, Glu-α H), 8.55 (br m, 2H, amide H).

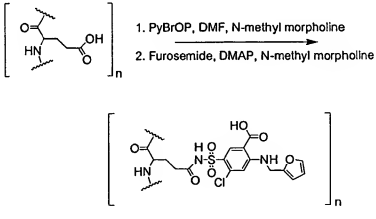
- Table 4: Percent of Active Agent Attached to a Carrier Peptide -

				% Active Agent	
Active Agent Conjugate	**Lot Number**	**Amount**	**Yield**	**NMR**	**UV**
pGlu(Stavudine)	TM112	1.00g	48%	35%	36%
pGlu(AZT)	TM113	1.35g	54%	41%	37%
pGlu(Fexofenadine) 1	TM46	10mg		21%	nd
pGlu(Fexofenadine) 2	TM132	300mg		56%	nd
pGlu(Lamivudine) 1A	TM114	~700mg		47%	nd
pGlu(Lamivudine) 1B	TM114	340mg		37%	nd
pGlu(Acetaminophen) 1	TM115	665mg	23%	37%	nd
pGlu(Acetaminophen) 2	TM143	1.00g	16%	14%	nd
pGlu(Zalcitabine)	TM119	190mg	24%	15-25%	24%
pGlu(Quetiapine)	TM120	700mg	34%	33%	43%
pGlu(Digoxin)	TM121	85mg	15%	33%	NA
pGlu(Dexamethasone)	TM123	225mg	29%	50%	nd
pGlu(Naltrexone) 1A	TM124	85mg		16%	nd

pGlu(Naltrexone) 1B	TM124	95mg		~16%	nd
pGlu(Metronidazole)	MA29	850mg	41%	40%	47%
pGlu(Azithromycin)	MA31	390mg	13%	33%	NA
pGlu(Simvastatin)	TM130	101mg	12%	45%	nd
pGlu(Atorvastatin) 1	TM128	53mg	5%	46%	nd
pGlu(Atorvastatin) 2	TM135	114mg	4%	22%	nd
pGlu(Tolteradine) 1	TM127	15mg	3%	15%	nd
pGlu(Tolteradine) 2	TM139	54mg	3%	5%	nd
pGlu(Tramadol) 1	TM129	15mg		29%	nd
pGlu(Tramadol) 2	TM138	187mg	6%	26%	nd
pGlu(Clavulanate)	TM134	320mg	48%	48%	nd
pGlu(Losartan)	TM144	1.81g	58%	55%	nd
pGlu(Raloxifene)	TM145	150mg	6%	40%	nd
pGlu(Naltrexone)	BBI152	404mg	26%	50%	55%
pGlu(Naltrexone)	BBI161	81mg	< 1%	40%	nd

nd: not determined; NA: not applicable.

III:M – Example: Attachment of Furosemide via Sulfonamide to the Side-chain of Poly(Glutamic Acid)



Reagents	Weight	MW	Molar Equivalents
1. poly(glutamic acid)	0.700 g	128 per residue	1 per residue

1. PyBrOP	2.04 g	466	0.8
1. N-methyl morpholine	1.26 mL	101	2.1
1. DMF	40 mL	-	-
2. Quetiapine	1.63 g	330.7	0.9
2. N-methyl morpholine	0.78 mL	101	1.3
2. DMAP	0.133 g	122	0.2

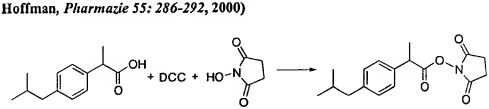
[207] To a solution of poly(glutamic acid) (0.700 g, 5.47 mmol) in DMF (40 mL) was added N-methyl morpholine (1.26 mL, 11.5 mmol) and bromo-*tris*-pyrrolidino-phosphonium hexafluorophosphate (PyBrOP) (2.04 g, 4.38 mmol). The resulting mixture was allowed to stir at room temperature for 30 minutes. After this time, N-methyl morpholine (0.78 mL, 7.11 mmol) and 4-dimethylaminopyridine (0.133 g, 1.09 mmol) (DMAP) followed by Furosemide (1.63 g, 4.92 mmol) were added. The resulting solution was stirred at room temperature for 24 hours. Solvent and excess base were then removed using reduced pressure evaporation. Water (100 mL) was then added and the resulting solid was collected and dissolved in saturated $NaHCO_3$. The crude product was purified using ultrafiltration. Product was then collected from ultrafiltration using acid precipitation as a green solid (0.678 g, 32%).

^{1}H NMR (360 MHz. DMSO-d_6): δ 1.84 (br d, 8H, Glu-β H), 2.273 (br m, 8H, Glu-γ H), 4.21 (br s, 4H, Glu-α H), 4.57 (br s, 2H, furosemide), 6.37 (br d, 2H, furosemide), 7.04 (br s, 1H, furosemide), 7.61 (br s, 1H, furosemide), 8.06 (br m, 4H, Glu NH), 8.51 (br s, 2H, furosemide), 8.80 (br s, 1H, furosemide), 12.25 (br s, 1.5H, Glu and Furosemide COOH).

Average % mass of Furosemide in poly-Glu(Furosemide): 39%.

III:N – Example: Synthesis of Poly-Lysine-Ibuprofen

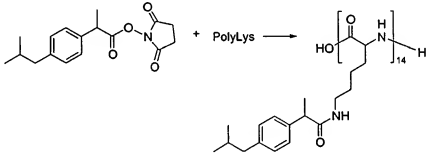
(i) Preparation of Ibuprofen-O-Succinimide (RI-172) (Grafe & Hoffman, *Pharmazie 55: 286-292*, 2000)



[208] To a stirring solution of ibuprofen (2.06 g, 10 mmol) in 5 mL of dioxane at room temperature was added a solution of dicyclohexylcarbodiimide (DCC, 2.27 g, 11 mmol) in 25 mL of dioxane. After 10 minutes a solution of N-hydroxysuccinimide (NHS, 1.16 g, 10 mmol) in 15 mL of dioxane was added. The reaction mixture was allowed to stir at room temperature for 5 hours and then filtered through a sintered glass funnel to remove the dicyclohexylurea (DCU). After rotary evaporation, the product was crystallized from methylene chloride/hexanes to yield 2.36 g (78%) of a colorless solid.

^{1}H NMR (500 MHz, DMSO-d6): δ 0.86 (d, 6, CH_3), 1.49 (d, 3,α- CH_3), 1.81 (m, 1, CH), 2.43 (d, 2, CH_2), 3.33 (m, 4, CH_2CH_2), 4.22 (q, 1, CH), 7.16 (d, 2, ArH), 7.28 (d, s, ArH).

(ii) Conjugation of Poly-Lysine with Ibuprofen-O-Succinimde (RI-197)



[209] Poly-lysine-HBr (Sigma, 100 mg, 34.5 nmol) was dissolved in 1 mL of water that had been brought to a pH of 8 with sodium bicarbonate, and stirred at room temperature. To this solution was added a solution of ibuprofen-O-succinimide (116 mg, 380 nmol) in 2 mL of dioxane. After stirring overnight, the dioxane was removed by rotary evaporation and diluted with 10 mL of pH 8 sodium bicarbonate in water. The precipitated product was filtered through a sintered glass funnel and washed with 3 x 10 mL of water and 4 x 10 mL of diethyl ether. After drying overnight by high vacuum the solid product was scraped out yielding 105 mg (62%).

^{1}H NMR (500 MHz, DMSO-d6): δ 0.85 (br s, 6, CH_3), 1.27 (br s, 3,α- CH_3), 1.40-1.79 (m, 5, CH of ibu and lysine γ and δ CH_2CH_2), 2.31 (d, 2, β CH_2), 2.41-2.52, under dmso (m, 2, β CH_2), 2.73-3.01 (m, 2, ε CH_2), 3.51-3.85 (m, 1 ibu CH), 4.01-4.43 (m, 1, α CH), 7.14 (d, 2, ArH), 7.6 (d, 2, ArH), 7.90-8.06 (m, 2, NH).

III:O – Example: Synthesis of PolyLysine-Naproxen

(i) Synthesis of Naproxen-Succinimide

[210] To Naproxen (2.303 g, 10 mmol) in 5 mL of dioxane was added N-hydroxysuccinimide (1.16 g, 10 mmol) dissolved in 15 mL of dioxane and dicyclohexylcarbodiimide (2.27 g, 11 mmol) in 25 mL of dioxane. The reaction was stirred overnight and the insoluble dicyclohexylurea removed by filtration. The solvent was removed by rotary evaporation and the residue dissolved in 30–40 mL CH_2Cl_2. Approximately 10 mL hexane was added and the mixture was chilled to 4°C for 2 hr. Additional hexane was added dropwise until small planar white crystals began to form and the solution was refrigerated overnight. The activated ester was harvested, washed with hexane and dried in vacuum (2.30 g, 70.0 %)

^{1}H NMR (500MHz, DMSO-d_6): δ 1.70 (d, 3H, CH_3) 2.9 (s, 4H, succinimide), 3.91 (s, 3H, OCH_3), 4.18 (q, 1H, methine) 7.75-7.12 (m, 6H, aromatic).

(ii) Synthesis of polylysine-Naproxen

[211] To $[Lys]_{14} \cdot 14 \cdot HBr$ (SEQ ID NO: 4) (0.100 g, 35 mmol) in 1 mL of H_2O (containing 10 mg/mL Na_2CO_3) was added Naproxen-Succinimide (0.124 g, 379 mmol) in 2 mL of dioxane. After stirring overnight a precipitate formed. More precipitate was formed by the addition of 30–40 mL of H_2O (containing 10 mg/mL Na_2CO_3), isolated by filtration and washed with 50 mL of Et_2O. The fine white powder was dried (0.095 g, 53%).

^{1}H NMR (500MHz, DMSO-d_6): δ 8.1 (m, 1H, lysine; amide), 7.8-7.0 (m, 6H, aromatic), 4.4-4.1 (m, 2H, α methine), 3.3 (s, 3H, OCH_3), 2.8 (m, 2H, ϵ), 1.7-1.0 (m, 9H, β, γ, δ, CH_3).

III:P – Example: Synthesis of PolyLysine-Gemfibrozil

(i) **Synthesis of Gemfibrozil – Succinimide**

[212] To Gemfibrozil (GEM) (5.0 g, 20.0 mmol) in 30 mL dioxane was added N- hydroxysuccinimide (2.3 g, 20.0 mmol) in 20 mL dioxane and dicyclohexylcarbodiimide (4.5 g, 22.0 mmol) in 50 mL dioxane. The reaction was stirred overnight and the insoluble dicyclohexylurea removed by filtration. The solvent was removed by rotary evaporation and the residue dissolved in 15 – 20 mL of CH_2Cl_2. Hexane was added dropwise until crystal formation was seen and the mixture was chilled to 4° C overnight. Approximately 3 mL of additional n-hexane was added and the mixture chilled to –20° C overnight. The activated ester formed small planer crystals and was harvested, washed with hexane and dried in vacuum (5.8 g, 80%).

^{1}H NMR (500 MHz, DMSO-d_6): δ 1.2, 1.3 (s, 6H, CH_3), 1.8-1.5 (m, 6H, GEM CH_2), 2.3-2.1 (s, 6H, aromatic CH_3) 2.85-2.7 (d, 4H, succinimide CH_2), 7.0-6.6 (m, 3H, aromatic).

(ii) **Synthesis of polylysine-Gemfibrozil**

[213] To $[Lys]_{11} \cdot 11$ HBr (SEQ ID NO: 5) (0.100 g, 43.5 μmol) in 1 mL of H_2O (containing 10 mg/mL Na_2C0_3) was added Gemfibrozil-succinimide (0.094 g, 261.1 μmol) in 2 mL dioxane. After stirring overnight a precipitate formed. More precipitate was formed by the addition of 30 mL of H_2O (containing 10 mg/mL Na_2C0_3), isolated and washed with 50 mL Et_2O. The fine white powder was dried (0.019 g, 1%).

^{1}H NMR (500 MHz, DMSO-d_6): δ 1.5-1.0 (m, 12H, β, γ, δ, CH_3), 1.85-1.5 (m, 4H, CH_2), 2.3, 2.1 (s, 6H, aromatic CH_3), 3.35 (s, 2H, ϵ), 3.85 (s, 2H, OCH_2), 4.05 (s, 1H, α), 5.6 (d, 1H, carbamate), 7.0-6.7 (m, 3H, aromatic), 8.0 (d, 1H, amide).

III:Q – Example: Preparation of PolyLysine Depakote (Valproic acid)

(i) **Synthesis of Valproic acid - Succinimide**

[214] To valproic acid (1.0 g, 6.9 mmol) in 14 mL 6:1 CH_2Cl_2:DMF was added N-hydroxysuccinimide (0.8 g, 6.9 mmol), dicyclohexylcarbodiimide (1.6 g, 7.6 mmol) and triethylamine (0.9 g, 8.9 mmol). The reaction was stirred for 60 h whereupon the solution was filtered to remove the white precipitate and the solvent

removed by rotary evaporation. The residue was purified by flash chromatography (10:1–2:1 hexane:EtOAc) to provide the succinimidyl ester as a clear oil (1.0 g, 59%).

R_f = 0.43 (3:1 hexane:EtOAc).

^{1}H NMR (300 MHz, $CDCl_3$): δ 2.76 (s, 4H, succinimide), 2.61 (m, 1H, methine), 1.65-1.19 (m, 8H, methylene), 0.88 (t, 6H, methyl).

(ii) Synthesis of PolyLysine-Valproic acid

[215] To Lys_{14}·HBr (SEQ ID NO: 4) (0.106 g, 37 μmol) in 0.8 mL H_2O pH 8 was added the valproic succinimidyl ester (0.104 g, 431 μmol) dissolved in 0.4 mL THF. The reaction was stirred overnight whereupon 8 mL H_2O was added. The mixture was acidified to pH 3 with 6 M HCl and extracted twice with 2 mL CH_2Cl_2. The aqueous layer was dried and the residue dissolved in 1 mL H_2O. The solution was purified by SEC (G-15, 10 mL dry volume) and eluted with water. Those fractions containing conjugate were combined and dried to yield a white solid (0.176 mg) which by ^{1}H NMR indicated 28 Lysine for every one drug molecule.

^{1}H NMR (300 MHz, D_2O): δ 4.29 (m, 1H, α), 3.00 (m, 2H, ε), 1.87-1.68 (m, 4H, β, δ), 1.43 (m, γ, methylene), 0.85 (t, methyl).

III:R – Example: Attachment of Pravastatin to the Side Chain of a Peptide

[216] The example below describes the preparation of $[Lys(Pravastatin)]_{15}$ where Pravastatin is attached to the side chain fo the peptide to form an mide linked drug/peptide conjugate.

[217] To Pravastatin sodium (0.994 g, 2226 μmol) in 10 mL dry DMF was added dicyclohexylcarbodiimide (0.458 g, 2226 μmol). After stirring for 3 hours under argon the solution was filtered through glass wool (to remove insoluble dicyclohexylurea) into a solution of polyLysine (0.288 g, 148 μmol) dissolved in 1 mL H_2O pH 8. After stirring overnight the milky white reaction was filtered through glass wool and the solvent removed by rotary evaporation. The resultant syrup was dissolved in 5 mL of 2-propanol and then diluted with 60 mL H_2O. The mixture was acidified to pH 6 with 1 N HCl and extracted thrice with 30 mL $CHCl_3$. The

organic solvent was removed by rotary evaporation and replaced with 45 mL H_2O and 5 mL 2-propanol. This solution was ultrafiltered (Amicon YM1, 1000 MW) with 100 mL H_2O and the retentate dried in vacuum to yield a white solid (130 mg).

R_f = 0.77 (6:1 $CHCl_3$:CH_3OH).

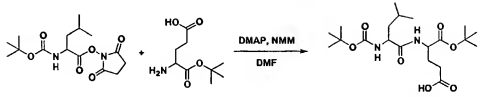
^1H NMR (500 MHz, DMSO-d_6): δ 5.97 (1H, 5'), 5.85 (1H, 3'), 5.50 (1H, 4'), 5.19 (1H, 8'), 4.78 (1H, 6'), 4.40 (1H, α), 4.15 and 4.01 (2H, 3, 5), 3.45 (2H, ε), 2.30 (4H, 2, 2, 2', 2"), 1.04 (3H, 2"-CH_3), 0.83 (6H, 4", 2'-CH_3).

III:S - Preparation of Boc-Leu-Ser(Boc-Ciprofloxacin)-OCH_3

[218] The solids Boc-Leu-Ser-OCH_3 (500 mg, 1.5 mmol), Boc-Cipro (970 mg, 2.25 mmol), PyBrop (1.05 g, 2.25 mmol) and DMAP (183 mg, 1.5 mmol) were dissolved in anhydrous DMF (15 ml). To this solution/ suspension was added N-methylmorpholine (414 µl, 3.75 mmol). The reaction mixture was then allowed to stir overnight at room temperature under argon. The majority of solvent was then removed under vacuum by rotary evaporation. The remaining oil was dissolved in $CHCl_3$ (20 ml), and the organic solution extracted initially with saturated $NaHCO_3$ (3.20 ml) and then with acidic water (pH3 HCl, 3.20 ml). The organic layer was collected and dried over $MgSO_4$. The suspension was then filtered and the $CHCl_3$ solution collected. The solvent was then removed under vacuum by rotary evaporation affording a yellow oil. This was redissolved in $CHCl_3$ (20 ml), and the organic solution extracted again with saturated $NaHCO_3$ (3x20 ml) and then with 0.1 N NaOH (3.20 ml). The organic solution was then dried over $MgSO_4$, and the suspension filtered with the $CHCl_3$ solution collected. The solvent was then removed under vacuum by rotary evaporation affording a yellow oil. this oil was dissolved in a minimal volume of $CHCl_3$ and the solution deposited on a preparative TLC plate. The plate was developed with 19:1 $CHCl_3$/MeOH. The second band was collected and characterized by ^1H-NMR. (500 MHz, $(CD_3)_2SO$) δ 0.868 (d, 6H, Leu_δ), 1.285-1.096 (m, 2H, Cip), 1.4-1.2 (m, 11H, Boc & Cip), 1.5-1.4 (m, 11H, Boc & Leu), 1.7-1.6 (m, 1H Leu_β), 3.3-3.1 (m, 4H, Cip), 3.6-3.5 (m, 4H, Cip), 3.660 (bs, 4H, -OCH_3 & Cip), 4.05-4.15 (m 1H, Leu_α), 4.35-4.5 (m, 2H, Ser_β), 4.7-4.8 (m, 1H Ser_α), 6.885 (d, 1H, amide), 7.489 (d, 1H, Cip), 7.79 (d, 1H, Cip) and 8.48 (d, 1H, Cip).

III: T - Preparation of Leu-Glu (AZT)

(i) Preparation of Boc-Leu-Glu-OtBu

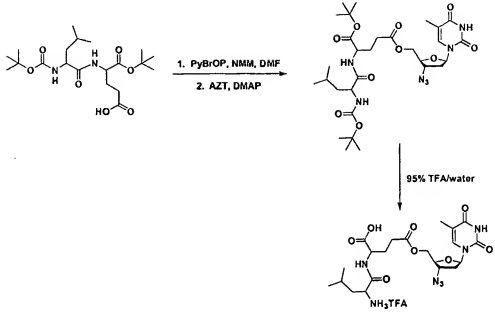


Reagents	MW	Weight	mmoles	Molar Equivalents
1. Boc-Leu-OSu	328	4.96g	15.1	1.0
1. Glu-OtBu	240	4.00g	16.6	1.1
1. DMAP	122	0.368g	3.02	0.2
2. NMM	101	2.07ml	18.9	1.25
2. DMF	-	50ml	-	-

Boc-Leu-Glu-OtBu

[219] To a solution of Boc-Leu-OSu and Glu-OtBu in DMF was added NMM and DMAP. The solution was stirred at ambient temperatures for 18 hours then the solvent was removed. Crude product was taken up in 100 ml $CHCl_3$, washed 3X with pH 3 water (100ml), once with water (100ml) and once with brine (100ml). Organic layer was dried with $MgSO_4$ and filtered. Solvent was removed and solid was dried over vacuum. Solid was collected as a white powder (8.78g, quant. yield, ~80% purity TLC). Product was used directly without further characterization.

(ii) Preparation of Leu-Glu(AZT)·TFA



Reagents	MW	Weight	mmoles	Molar Equivalents
1. Boc-Leu-Glu-OtBu	416	4.39g	10.6	1.0
1. PyBrOP	466	7.49g	16.1	1.5
1. DMF	-	50ml	-	-
1. NMM	101	3.53ml	32.2	3.0
2. AZT	267	2.86g	10.7	1.0
2. DMAP	122	0.261g	2.14	0.2

Boc-Leu-Glu-OtBu

[220] To a solution of Boc-Leu-Glu-OtBu in DMF was added NMM and PyBrOP. The solution was stirred at ambient temperatures for 0.5 hours then the AZT and DMAP were added. The reaction mixture was then stirred at ambient temperatures for 18 hours. Solvent was removed and crude product was taken up in 400 ml $CHCl_3$, washed 3X with pH 3 water (250ml), once with water (250ml) and once with brine (250ml). Organic layer was dried with $MgSO_4$ and filtered. Solvent was removed and solid was dried over vacuum. Product was purified using prep. HPLC [Phenomenex Luna C18, 30X250mm, 5μM, 100Å; Gradient: (100 0.1% TFA–water/0 0.1% TFA-MeCN → 80/20) 0-10 min. (80/20 → 50/50) 10-25 min.;

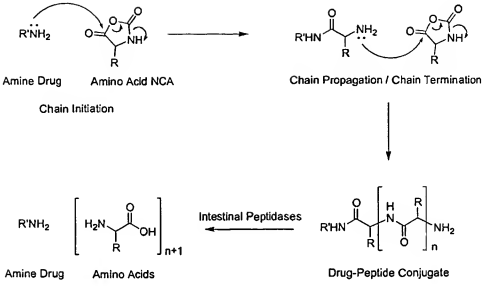
30ml/min.]. Product was deprotected using 100ml 95%TFA/water. Product was dried over a vacuum to give a slightly yellow solid (2.45g, 4.04mmoles, 38% yield): ^{1}H NMR (DMSO-d_6) δ 0.90 (m, 6H), 1.58 (m, 2H), 1.69 (m, 2H), 1.80 (s, 3H), 1.87 (m, 1H), 2.07 (m, 1H), 2.35 (m, 1H), 2.45 (m, 2H), 3.78 (m, 1H), 3.97 (t, 1H), 4.28 (m, 3H), 4.45 (t, 1H), 6.14 (t, 1H), 7.46 (s, 1H), 8.14 (br s, 3H), 8.75 (d, 1H), 11.32 (br s, 1H).

IV Amino Acid Active Agents and their Attachment to a Peptide

[221] Amino acid active agents allow for a distinct embodiment of the present invention. This embodiment occurs when the active agent itself is an amino acid and thus capable of both amine and carboxylate bonding allowing for the interspersement of the active agent into the peptide chain. Amino acid drugs can be C-capped, N-capped and sidechain attached according to the procedures and mechanisms described above. As a unique aspect to amino acid drugs, the active agent may be interspersed within the peptide chain through a peptide bond at both ends of the active agent.

[222] Most preferred drug conjugates for the interspersed attachment of amino acid active agents include amoxicillin; amoxicillin and clavulanate; baclofen; benazepril hydrochloride; candoxatril; captopril; carbidopa/Levodapa; cefaclor; cefadroxil; cephalexin; cilastatin sodium and imipenem; ciprofloxacin; diclofenac sodium; diclofenac sodium and misoprostol; enalapril; enapril maleate; gabapentin; levothyroxine; lisinopril; lisinopril and hydroichlorothiazide; loracarbef; mesalamine; pregabalin; quinapril hydrochloride; sitafloxacin; tirofiban hydrochloride; trandolapril; and trovafloxacin mesylate.

[223] An example of interspersed attachment conjugation to an amino acid drug is depicted in the scheme below:



R=Side chain of amino acid or peptide
R'=Radical moiety attached to amine functionality on drug
NCA=N-carboxyanhydride

IV:A – Preparation of Active Agent Conjugates (N-terminus)

[224] In the following examples the amino acid active agent is attached to the N-terminus of different amino acids. These examples do not only describe an N-terminus attachment, but also represent the peptide linkage of the amino acid active agent as an amino acid to form either a dipeptide or a peptide conjugate.

[225] T4 conjugated to amino acid polymers were either prepared by coupling (protected) T4 to commercially available amino acid homopolymers or incorporated by addition of T4-NCA to the corresponding polypeptide in situ.

(i) T4 Conjugation to a Commercial Polyglycine

[226] To N-TeocT4 (0.017 g, 17 μmol) in 1 mL dry DMF was added dicyclohexylcarbodiimide (0.004 g, 18 μmol). After stirring for 30 minutes N-dimethyl-4-aminopyridine (0.004 g, 36 μmol) and Gly_{18} (SEQ ID NO: 6) (0.017 g, 17 μmol) were added and the reaction stirred overnight. The cloudy solution was poured into 20 mL H_2O and extracted twice with 10 mL CH_2Cl_2. The aqueous

component was acidified to pH 3 with 1 N HCl and chilled to 4°C. The material was isolated by centrifugation and the pellet washed 3 times with 8 mL H_2O. The pellet was dried in vacuum to yield dicyclohexylurea and N-TeocT4-Gly_{18} (SEQ ID NO: 6).

^{1}H NMR (500 MHz, DMSO-d_6): δ 7.8 (T4 aromatic), 7.1 (T4 aromatic), 4.1 (α).

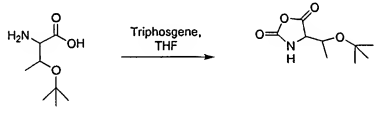
[227] To the impure protected polymer was added 2 mL trifluoroacetic acid. The reaction was stirred for 2 hours at room temperature and the solvent removed by rotary evaporation. The residue was dissolved in 1 mL DMF and the insoluble material removed by filtration. The DMF was removed by rotary evaporation and dried in vacuum to yield a white material (.012 g, 40%).

^{1}H NMP (500 MHz, DMSO-d_6): δ 7.75 (T4 aromatic), 7.08 (T4 aromatic), 4.11 (bs, α).

(ii) **Preparation of Amino Acid-NCA**

[228] N-carboxyanhydrides (NCA's) of the amino acids listed below were prepared using a protocol similar to that reported for glutamic acid. Minor variations in their final workups are noted below.

Thr-NCA



[229] To a mixture of Thr-OtBu (0.500 g, 2.85 mmol) in THF (25mL) was added triphosgene (0.677 g, 2.28 mmol). The resulting solution was stirred at reflux for 3 hours. The solution was evaporated to dryness to obtain Thr-NCA (0.500 g, 2.48 mmol, 87%) as a white solid. Thr-NCA was used without further characterization.

[230] To the L-amino acid (1.5 g) in 100 mL dry THF was added triphosgene (0.8 eqv). The reaction was vessel was equipped with a reflux condenser and NaOH trap and heated to reflux for 3 h. The solvent was removed by rotary evaporation and the residue washed with hexane to yield the amino acid NCA as white residue.

Leu-NCA: ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.65 (s, 1H, NH), 4.33 (dd, 1H, α), 1.82 (m, 2H, β), 1.68 (m, 1H, γ), 0.98 (dd, 6H, δ).

Phe-NCA: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.36-7.18 (m, 5H), 5.84 (s, 1H, NH), 4.53 (dd, 1H), 3.28 (dd, 1H, α), 2.98 (dd, 1H, β).

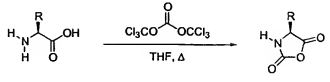
Trp(Boc)-NCA: ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.14 (d, 1H), 7.49 (d, 2H), 7.36 (t, 1H), 7.27 (m, 1H), 5.90 (s, 1H, NH), 4.59 (dd, 1H, α), 3.41 (dd, 1H, β), 3.07 (dd, 1H, β), 1.67 (s, 9H, t-Bu).

Ile-NCA: ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.65 (s, 1H, NH), 4.25 (d, 1H, α), 1.94 (m, 1H, β), 1.43 (dm, 2H, γ-CH_2), 1.03 (d, 3H, γ-CH_3), 0.94 (t, 3H, δ).

Lys(Boc)-NCA: 1H NMR (500 MHz, CDCl3): □ 6.65 (bs, 1H, NtH), 4.64 (s, 1H, carbamate NH), 4.31 (t, 1H, α), 3.13 (s, 2H, ϵ), 2.04 (m, 2H, β), 1.84 (m, 2H, δ), 1.48 (m, 11H, γ, t-Bu).

Met-NCA: ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.89 (s, 1H, NH), 4.50 (dd, 1H, α), 2.69 (t, 2H, γ), 2.10 (m, 1H, β), 2.08 (m, 4H, β, δ).

(iii) Example of Other Amino Acid-NCA's



tButyl Aspartic acid R =

Valine R =

tButyl Serine R =

tButyl Glutamic Acid R =

Proline R =

5

- Table 5: Amino Acids for use as NCA's for Peptide Synthesis –

Amino Acid	Chemical Shift in the NCA α	β	γ	Other (OtBu)
Alanine	4.41 (q, 1H)	1.57 (d, 3H)		
Valine	4.20 (d, 1H)	2.28-2.19 (m, 1H)	1.08 (d, 3H) 1.02 (d, 3H)	
Serine (OtBu)	4.58 (m, 1H)	3.62 (dd, 1H) 3.50 (dd, 1H)		1.10 (s, 9H)
Aspartic acid (OtBu)	4.51 (dd,1H)	2.93 (dd, 1H) 2.73 (dd, 1H)		1.44 (s, 9H)
Glutamic acid (OtBu)	) 4.34 (dd,1H)	2.28-2.20 (m, 1H) 2.09-1.99(m, 1H)	2.45 (t, 2H)	1.44 (s, 9H)

10

- Table 6 -

Amino Acid	Isolation of NCA
Alanine	precipitate with hexanes in 68% yield
Valine	precipitate with hexanes in 89% yield
Serine (OtBu)	suspended in isopropanol and precipitated with hexanes in 83% yield
Aspartic acod (OtBu)	suspended in isopropanol and precipitated with hexanes in 55% yield
Glutamic acid (OtBu)	suspended in isopropanol and precipitated with hexanes in 77% yield

(iv) **T4 Conjugation to Preformed Homopolymer in situ**

T4-Asp$_n$

[231] The below example depicts the attachment of T4 to the N-terminus of peptide conjugate polyAsp in situ. Polyserine and Polythreonine were also prepared using this protocol. The serine reaction mixture contained N-methylmorpholine (1.1 equivalents).

[232] Polyaspartic acid: Asp(OtBu) (13 mg, 0.07 mmol) and Asp(OtBu)-NCA (200 mg, 0.93 mmol) were dissolved in anhydrous DMF (5 mL), and the solution allowed to stir over night at room temperature under argon. The following morning, 2.5 mL of the reaction mixture was transferred to separate flask (Flask B). T4-NCA (27 mg, 0.03 mmol) was added to the original flask (Flask A), and both solutions were allowed to continue stirring under argon for an additional 24 hours. Polymer was then precipitated by the addition of water (50 mL) to each flask. The resulting solids were collected by filtration and dried over night under vacuum.

[233] The dried Asp(OtBu)$_n$ (Flask B) and T4-Asp(OtBu)$_n$ (Flask A) were then dissolved in 95% trifluoroacetic acid in water (3 mL) and allowed to stir at room temperature for 2 hours. The deprotected polymers were then precipitated by the addition of ethyl ether (10 mL) and then storing the suspension at 4 °C for 2 hours. The respective polymers were then collected by filtration and the solids dried over night under vacuum. This afforded 48 mg of Asp$_n$ (Flask B) and 12 mg of T4-

Asp_n (Flask A). MALDI indicated that T4-Asp_n (Flask A) consisted of a mixture of polymers of varying lengths: T4-Asp_{3-12} (SEQ ID NO: 7).

- Table 7: Amino Acid Conjugates of T4 -

Amino acid derivative	Polymer	Isolated	Percent yield	Mass Range
Asp(OtBu)	$Asp(OtBu)_n$	48 mg	84%	NA
	T4-$Asp(OtBu)_n$	12 mg	14%	T4-Asp_{3-12} (SEQ ID NO: 7)
	12mg	14%		
Ser(OtBu)	$Ser(OtBu)_n$	73mg	101%[3]	Ser_{7-8} (SEQ ID NO: 20)
	T4-$Ser(OtBu)_n$	50mg	43%	T4-Ser_{4-9} (SEQ ID NO: 21)
Thr(OtBu)	$Thr(OtBu)_n$	29mg	20%	Thr_{7-8} (SEQ ID NO: 22)
	T4-$Thr(OtBu)_n$	66mg	24%	T4-Thr_{1-8} (SEQ ID NO: 23)

[3]: The percent yield was estimated based on the total amino acid content in the original reaction prior to splitting the reaction. The Mass Range was determined from MALDI. The yield over 100% could reflect either the presence of salts or uneven distribution when the reaction mixture was split.

Other examples of T4 Conjugation to Preformed Homopolymer in situ are listed below:

T4-Leu_{15} (SEQ ID NO: 8)

[234] To IleNCA (0.200 g, 1.3 μmol) in 2.5 mL DMF was added isoleucine (0.012 g, 0.1 μmol). After stirring overnight under Ar T4-NCA (0.037 g, 0.050 μmol) was added and the reaction stirred an additional 72 hours. The white solution was added to 8 mL H_2O. The heterogeneous solution was chilled to 4°C, centrifuged and the supernatant discarded and the pellet washed with 8 mL H_2O. The dried residue was washed with 50 mL ethanol warmed to 50°C to yield after drying, a white powder (0.124 g, 55%).

^{1}H NMR (500MHz, DMSO-d_6): δ 7.75 (s, T4 aromatic), 7.08 (s, T4 aromatic), 4.11 (dd, α), 1.77 (m, β), 1.38 (m, β, γ-CH), 0.91 (m, γ-CH, γ-CH_3, δ).

T4-Phe_{15} (SEQ ID NO: 9)

White powder (58%).

^{1}H NMR (360 MHz, DMSO-d_6): δ 7.0-8.1 (NH, aromatics), 4.5 (α), 3.0 (β); MALDI-MS indicates T4-Phe_{15} (SEQ ID NO: 9).

T4-Met_{15} (SEQ ID NO: 10).

White powder (10%).

^{1}H NMR (500MHz, DMSO-d_6): δ 8.0-8.5 (amide NH), 4.4 (α) 2.5 (γ), 2.05 (ε), 2.0-1.7 (β).

T4-Val_{15} (SEQ ID NO: 11)

White powder (14%).

^{1}H NMP (500MHζ, DMSO-d_6): δ 7.75 (T4 aromatic), 7.08 (T4 aromatic), 4.35 (α), 3.45 (β), 1.05 (γ).

For those conjugates that used a protected NCA an additional, separate deprotection step was necessary:

T4-Lys_{4-11} (SEQ ID NO: 12)

[235] To T4-[Lys(Boc)]$_{4-11}$ (SEQ ID NO: 12) (0.256 g, 61 μmol) in 10 mL of CH_2Cl_2 was stirred with trifluoroacetic acid (10 mL) for 2 h. The solvent was removed by rotary evaporation and the residue dissolved in 3 mL H_2O and ultrafiltered (Amicon regenerated cellulose, YM1, NMWL 1000, wash with 30 mL pH 5 H_2O). The retentate was dried in vacuum to give a light brown residue.

^{1}H NMP (500MHz, D_2O): δ 7.82 (s, T4 aromatic), 7.41 (s, T4 aromatic), 4.29 (bs, α), 3.00 (bs, ε), 2.13-1.70 (m, β, δ, γ); MALDI-MS gives a range T4-Lys_{4-11} (SEQ ID NO: 12).

T4-Trp_{15} (SEQ ID NO: 13)

^{1}H NMR (500MHz, DMSO-d_6): δ 8.25-6.80 (m, aromatic), 4.50 (bs, α), 3.40 (bs, β), 3.00 (bs, β).

IV:B - Preparation of Active Agent Conjugates (C-terminus)

(i) **Typical preparation of T4 C-capped homopolymers:**

Trp_{15}-T4 (SEQ ID NO: 13)

[236] To T4 (0.078 g, 100 μmol) in 10 mL dry DMF was added Trp(Boc)-NCA (0.500 g, 1.514 mmol). After stirring for 64 hours under argon the reaction was quenched by adding 30 mL H2O. The cloudy white solution was chilled to 4°C, centrifuged and the pellet washed three times with 25 mL H2O. The residue was dried in vacuum to provide $Trp(Boc)_{15}$-T4 (SEQ ID NO: 13) as a brown solid. This material was further purified by ultrafiltration (Amicon regenerated cellulose, YM1, NMWL 1000, wash with 30 mL pH 5 H_2O) to provide $[Trp(Boc)]_{15}$-T4 (SEQ ID NO: 13) as a brown-gold solid (0.400 g, 79%).

^{1}H NMP (500 MHz, DMSO-d_6): δ 8.25-6.80 (m, aromatic), 4.50 (bs, α), 3.40 (bs, β), 3.00 (bs, β), 1.50 (bs, t-Bu).

[237] To $[Trp(Boc)]_{15}$-T4 (SEQ ID NO: 13) (0.509 g) in 8 mL of 1:1 CH_2Cl_2:trifluoroacetic acid was stirred for 1.5 hours. The solvent was removed by rotary evaporation and the residue dried in vacuum to yield a brown solid (0.347 g, 97%).

^{1}H NMP (500 MHz, DMSO-d_6): δ 8.25-6.80 (m, aromatic), 4.50 (bs, β), 3.40 (bs, α), 3.00 (bs, β).

Lys_{15}T4 (SEQ ID NO: 14)

[238] Lys_{15}-T4 (SEQ ID NO: 14) was prepared using a similar protocol than the one used for Trp_{15}-T4 (SEQ ID NO: 14).

$[Lys(Boc)]_{15}$-T4: (SEQ ID NO: 14)

^{1}H NMR (500MHz, D_2O): δ 7.82 (s, T4 aromatic), 7.41 (s, T4 aromatic), 4.29 (bs, α), 3.00 (bs, ε), 2.13-1.70 (m, β, δ, γ).

Lys_{15}-T4: (SEQ ID NO: 14)

^{1}H NMR (500MHz, D_2O): δ 7.82 (s, T4 aromatic), 7.41 (s, T4 aromatic), 4.29 (bs, α), 3.00 (bs, ε), 2.13-1.70 (m, β, δ, γ).

(ii) **Synthesis of $[Glu]_{15}$ - L-dihydroxyphenylalanine** (SEQ ID NO: 3) **or $[Glu]_{15}$-L-DOPA** (SEQ ID NO: 3)

[239] L-DOPA (0.050 g, 254 μmol) and GluNCA (0.666 g, 3.85 mmol) were dissolved in 6 mL DMF. After stirring overnight under Argon, the reaction was examined by thin layer chromatography (9:1 H_2O: HOAc) showed some free drug (R_f = 0.70) and a more polar spot presumed to be polymer (R_f = 0.27). The reaction was quenched by the addition of 12 mL H_2O. The pH was adjusted to pH 1-2 using 1N HCl. The solvent was removed by rotary evaporation and the viscous residue dried in vacuum. The resultant syrup was transferred to a new vessel in H_2O and lyophilized. The resulting crystals were off white to light brown. Yield: 0.470 g, 62%. ^1H NMR showed pyroglutamic acid contamination; therefore, the material was suspended in H_2O and ultrafiltered (Millipore, regenerated cellulose, YM1, NMWL =1000), and the retentate dried under vacuum. Yield: 0.298 grams.

^1H NMR (500MHz, DMSO-d_6) indicated a relative ratio of 30:1 Glu:L-DOPA: δ 6.6 (L-DOPA aromatic), 6.4 (L-DOPA aromatic), 4.1 (Glu, α), 1.85 (Glu, β), 2.25 (Glu, γ, L-DOPA), 2.3 (L-DOPA, benzylic), 12.4-11.5 (Glu, CO_2H), 8.0 (Glu, amide)

(iii) **Synthesis of $[Glu]_{10}$-L-DOPA** (SEQ ID NO: 15)

[240] As in the synthesis of $[Glu]_{15}$-L-DOPA (SEQ ID NO: 3) except 0.439 grams of GluNCA were used. The final yield of purified material was 0.007 grams.

The ^1H NMR (500MHz, DMSO-d_6) indicates 8:1 Glu:L-DOPA.

IV;C - Preparation of Interspersed Active Agent/Peptide Conjugates

(i) **Synthesis of Random Copolymer of T4 and Trp**

[241] To T4-NCA (0.065 g, 0.1 mmol) and Trp(Boc)-NCA (0.400 g, 1.2 mmol) were combined in 4 mL dry DMF. Triethylamine (11 μl, 0.1 mmol) was added and the reaction stirred for 44 hours under argon. After quenching by the addition of 10 mL H_2O the heterogeneous mix was chilled to 4°C and centrifuged. The pellet was isolated and washed three times with 10 mL H_2O and dried in vacuum.

[242] To the random T4/[Trp(Boc)]$_{15}$ (SEQ ID NO: 13) polymer was added 10 mL 1:1 CH_2Cl_2:trifluoroacetic acid and the reaction stirred for 1 hour. The solvent was removed by rotary evaporation to provide the deprotected polymer as a brown solid (0.262 g, 91%) which was further purified by ultrafiltration (Amicon regenerated cellulose, YM1, NMWL 1000, wash with 30 mL pH 5 H_2O).

^{1}H NMR (500MHz, DMSO-d_6): δ 8.25-6.80 (m, aromatic), 4.50 (bs, α), 3.40 (bs, β), 3.00 (bs, β).

(ii) **Synthesis of Random Copolymer of T4 and Lys**

[243] Random T4/Lys$_{15}$ (SEQ ID NO: 14) was prepared using a protocol similar to the one used to prepare Random T4/Trp.

^{1}H NMR (500MHz, D_2O): δ 7.82 (s, T4 aromatic), 7.41 (s, T4 aromatic), 4.29 (bs, α), 3.00 (bs, ε), 2.13-1.70 (m, β, δ, γ).

IV:D - Fatty Acid Acylation

(i) **Preparation of N-Palmitoyl-L-triiodothyronine (C16T3)**

[244] To palmitic acid (0.500 g, 2.0 mmol) in 5 mL of dichloromethane, CH_2Cl_2, was added DCC (0.201 g, 1.0 mmol). The solution was allowed to stir for 45 minutes whereupon it was filtered through glasswool to remove insoluble 1,3-dicyclohexylurea, DCU, into 3 mL of DMF containing L-triiodothyronine (0.578 g, 0.9 mmol) and N-dimethyl-4-aminopyridine (0.119 g, 1.0 mmol). After stirring for 18h the solvent was removed by rotary evaporation and the residue purified by flash chromatography (30:1-8:1 $CHCl_3$:CH_3OH with 1 drop HOAc/100 mL eluent) to provide the target as a white solid (0.242 g, 31%): R_f (6:1 $CHCl_3$:CH_3OH) 0.27; ^{1}H NMR ($CDCl_3$ 500 MHz) 8.10 (d, 2H), 7.63 (s, 1H, NH), 7.06-6.48 (m, 3H), 4.64 (bs, 1H, α), 3.12 (m, 2H, β), 2.16 (m, 2H), 1.55 (m, 2H), 1.33-1.10 (bs, 24H), 0.83 (t, 3H).

(ii) **Prepartion of N-Octanoyl-L-triiodothyronine(O-octanoyl) (C8T3(C8))**

[245] To octanoic acid (0.30 mL, 1.9 mmol) in 5 mL of CH_2Cl_2 was added DCC (0.201 g, 1.0 mmol). The solution was allowed to stir for 30 minutes whereupon it was filtered through glasswool to remove insoluble DCU into 3 mL of DMF containing L-triiodothyronine (0.578 g, 0.9 mmol) and N-dimethyl-4-aminopyridine (0.217 g, 1.8 mmol). After stirring for 16h the solvent was removed by rotary evaporation and the residue purified by flash chromatography (30:1-8:1 $CHCl_3$:CH_3OH with 1 drop HOAc/100 mL eluent) to provide the target as a white solid (0.473 g, 64%): R_f (6:1 $CHCl_3$:CH_3OH) 0.16; ^1H NMR ($CDCl_3$ 500 MHz) 8.16 (d, 2H), 7.65 (s, 1H, NH), 7.11-6.52 (m, 3H), 4.68 (dd, 1H, α), 3.17 (dd, 1H, β), 3.08 (dd, 1H, β), 2.28 (m, 4H), 1.61 (m, 4H), 1.29-1.20 (bs, 16H), 0.85 (m, 6H).

(iii) Prepartion of Triiodothyronine Octanoate - TFA (T3C8)

a) To TeocT3 (0.300 g, 0.38 mmol) in 3 mL of dry DMF was added DCC (0.086 g, 0.42 mmol), 1-octanol (0.2 mL, 1.2 mmol) and N-dimethyl-4-aminopyridine (0.051 g, 0.42 mmol). After stirring for 21h the solvent was removed by rotary evaporation and the residue purified by flash chromatography (12:1-0:1 hexane:EtOAc) to provide the target as a white solid (0.187 g, 55%): R_f (1:1 hexane:EtOAc) 0.95; ^1H NMR ($CDCl_3$ 500 MHz) 7.62 (s, 2H), 7.11-6.57 (m, 3H), 5.29 (d, 1H, NH), 4.57 (m, 1H, α), 4.08 (m, 4H, C(O)OCH_2alkyl, $CH_2OC(O)N$), 2.88 (m, 2H, β), 2.28 (m, 4H), 1.57 (m, 2H, OCH_2CH_2alkyl), 1.30-1.24 (m, 10H), 0.96 (m, 2H, $SiCH_2$), 0.84 (m, 3H, CH_3).

b) TeocT3C8 material (0.187 g, 0.21 mmol) was dissolved in 10 mL of CH_2Cl_2 and 5 mL of trifluoroacetic acid, TFA. After stirring for 1h the solvent was removed by rotary evaporation target as a white solid (0.177 g, 100%): R_f (1:1 hexane:EtOAc) 0.78; ^1H NMR (DMSO 500 MHz) 7.83 (s, 2H), 6.95-6.66 (m, 3H), 4.45 (m, 1H, α), 4.13 (m, 2H, C(O)OCH_2alkyl), 3.30 (m, 1H, β), 3.06 (m, 1H, β), 2.00 (m, 2H), 1.52 (m, 2H), 1.30-1.25 (m, 10), 0.86 (m, 3H, CH_3).

(iv) Preparation of Trioodothyronine hexadeconoate - TFA (T3C16)

a) To TeocT3 (0.300 g, 0.38 mmol) in 3 mL of dry DMF was added DCC (0.086 g, 0.42 mmol), 1-hexadecanol (0.274 g, 1.13 mmol) and N-dimethyl-4-

aminopyridine (0.051 g, 0.42 mmol). After stirring for 18.5h the solvent was removed by rotary evaporation and the residue purified by flash chromatography (12:1-0:1 hexane:EtOAc) to provide the target as a white solid contaminated with 1-hexadecanol (0.348 g, 90%): R_f (3:1 hexane:EtOAc) 0.46; ^{1}H NMR ($CDCl_3$ 500 MHz) 7.63 (s, 2H), 7.08-6.62 (m, 3H), 5.29 (d, 1H, NH), 4.56 (m, 1H, α), 4.11 (m, 4H, C(O)OCH_2alkyl, $CH_2OC(O)N$), 2.99 (m, 2H, β), 2.28 (m, 4H), 1.55 (m, 2H, OCH_2CH_2alkyl), 1.31-1.24 (m, 26H), 0.96 (m, 2H, $SiCH_2$), 0.86 (m, 3H, CH_3).

b) The impure TeocT3C16 material (0.348 g) was dissolved in 10 mL of CH_2Cl_2 and 5 mL of TFA. After stirring for 1h the solvent was removed by rotary evaporation target as a white solid: R_f (1:1 hexane:EtOAc) 0.85; ^{1}H NMR (DMSO 500 MHz) 7.84 (s, 2H), 6.95-6.64 (m, 3H), 4.45 (m, 1H, α), 4.10 (m, 2H, C(O)OCH_2alkyl), 3.30 (m, 1H, β), 3.06 (m, 1H, β), 2.00 (m, 2H), 1.52 (m, 2H), 1.30-1.25 (m, 10), 0.86 (m, 3H, CH_3).

IV:E - Synthesis of mPEG-Amine-Triiodothyronine

(i) Synthesis of mPEG-Teoc-T3

[246] To a stirring solution of Teoc-T3 (88 mg, 0.11 mmol) in 3 mL of dry DMF under Ar was added DCC (25 mg, 1.20 mmol). After stirring overnight the insoluble DCU was filtered and the solid byproduct was washed with 2 mL of DMF. To the combined clear filtrates was added mPEG-amine (534 mg, 0.10 mmol, average MW = 5336) and 3 mL additional DMF. The solution was heated briefly with a heat gun until all of the amine was dissolved. The reaction was allowed to stir at room temperature overnight. The reaction solution was poured into 50 mL of diethylether causing the product to crash out as a white solid which was filtered. The solid product was then dissolved into 10 mL of DMF and poured into 50 mL of ether once again. This process was repeated one additional time and the filtered solid was dried by high vacuum overnight yielding 340 mg (56%) of the hygroscopic product.

(ii) Deprotection of mPEG-Teoc-T3

[247] The dried product from part A was stirred in 3 mL of TFA at room temperature for one hour. The TFA was removed by rotary evaporation of the azeotrope with hexane. The residue was dissolved in 3 mL of DMF and this solution was poured into 50 mL of ether. This suspension was cooled to 4°C, filtered and dried by high vacuum for 5 hours. This material was further purified by ultrafiltration (3,000 MW) filter using saturated sodium bicarbonate as a diluent. The product was dissolved in 10 mL of diluent and passed through the filter at 40 psi the rinsed in a similar manner 4 times. The residue was taken up in 3 mL of water and the filter was rinsed two additional times with 3 mL of water. The combined solution was frozen and lyophilized resulting in 162 mg (55%) of a fluffy white powder. T3 quantity present in conjugate by UV potency (λ_{320}, 1 M NaOH) was determined to 5.3% of total mass.

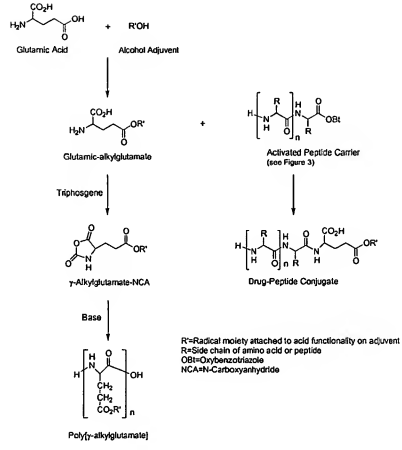
IV:F - Preparation of Triiodothyronine Cyclodextrin Ester

[248] To TeocT3 (0.457 g, 0.57 mmol) in 5 mL dry DMF was added DCC (0.237 g, 1.15 mmol). After stirring for 40 min under Ar was added β-cyclodextrin (0.652 g, 0.57 mmol) and N-dimethyl-4-aminopyridine (0.070 g, 0.57 mmol). After stirring the suspension for 26 h under Ar 20 mL H2O was added. The cloudy white solution was filtered through glasswool and washed with 20 mL EtOAc. The water was removed by lyophilization and the off white residue purified by flash chromatography (C18 CH_3OH) to provide roughly a 1:1 mixture of TeocT3-β-CD (R_f 7:7:5:4 EtOAc:2-propanol:NH_4OH:H_2O) 0.64) and unmodified β-CD (R_f 0.28) as an off-white solid (0.098 g).

V General Prepartion of Peptide Adjuvants

[249] While specific examples of active agents describe how to make different embodiments of the invention the general prepartion of amino acid conjugates are decribed below and may be used in combination or alone with any number of active agents. Formation of the peptide portion of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples, or other known techniques.

Alcohol Adjuvent/Glutamic Acid Dimer Preparation and Conjugation Scheme



V:A - Adjuvant Attachment of Side-Chain Carrier Peptide

(i) Preparation of γ-Alkyl Glutamate

[250] In reference to the above scheme, there have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

(ii) Preparation of γ-Alkyl Glutamate-NCA

[251] γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

(iii) Preparation of Poly[γ-Alkyl Glutamate]

[252] γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

(iv) γ-Alkyl Glutamate/C-Terminus Conjugation

[253] Again in reference to the above scheme, the peptide carrier can be dissolved in DMF under nitrogen and cooled to 0 ^{o}C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

V:B - Specific Example of Preparation of Poly-γ-Benzylglutamic Acid

(i) Preparation of benzylglutamic acid-NCA (adjuvant)

[254] Benzylglutamic acid (25 grams) was suspended in 400 mL anhydrous ethyl acetate under nitrogen. The mixture was heated to reflux where 30 grams of triphosgene was added in six (6) equal portions. The reaction was refluxed for three (3) hours until homogenous. The solution was cooled to room temperature, filtered, and concentrated *in vacuo*. The white powder was recrystallized from 50 mL of hot anhydrous ethyl acetate to yield 17.4 grams (63%) of a white powder.

(ii) Preparation of polybenzylglutamic acid

[255] Benzylglutamic acid (17.4 grams) was dissolved in anhydrous tetrahydrofuran (THF) under nitrogen where 238 mg of sodium methoxide was added portion wise. The solution was stirred for two (2) days with a marked increase in viscosity. The solution was poured into 1.5 L of petroleum ether with stirring. The petroleum ether was decanted off and an additional 1L of petroleum ether was added back. The mixture was stirred by hand, the petroleum ether was decanted off and the process repeated with 500 mL of petroleum ether. The white solid was air-dried and then vacuum dried to yield 14.7 (95%) of a white fluffy paper-like solid.

V:C - Preparation of Various Peptides

(i) Preparation of Polyglutamic Acid

[256] 1.96 g of Polybenzylglutamic acid added by hand was stirred in 10 mL of 30wt% hydrogen bromide (HBr) in acetic acid. The mixture was stirred at room temperature for one day and was, then, added to 50 mL of ether. The white precipitant was filtered, washed with 4 x 30 mL of ether and dried under a high vacuum to yield 1.11 grams (97%) of a white powder.

(ii) Preparation of Polyarginine

[257] All reagents were used as received. ^{1}H NMR was run on a Bruker 300 MHz (300) or JEOL 500 MHz (500) NMR spectrophotometer using tetramethylsilane as an internal standard. Thin layer chromatography was performed using plates precoated with silica gel 60 F_{254}. Flash chromatography was performed using silica gel 60 (230-400 mesh).

(a) Method 1

[258] To H-Arg(Z) $_2$-OH (0.300 g, 0.68 mmol) in 3.0 mL dry DMSO was added diphenylphosphorylazide (219 μl, 1.02 mmol) and triethylamine (236 μl, 1.69 mmol). The reaction was stirred for 48 hours under argon upon which the solution was poured into 100 mL H_2O. The resulting heterogeneous solution was centrifuged to isolate the white precipitate which was washed 3 x 100 mL H_2O, 3 x 100 mL CH_2OH and 100 mL Et_2O and then vacuumed dried to obtain 172 mg of an off white solid.

^{1}H NMR (500 MHz, DMSO-d_6): δ 7.31 (m, 10H), 5.21 (m, 1H, benzylic), 5.01 (m, 1H, benzylic), 3.83 (m, 1H, α), 3.34 (m, 2H, δ) 1.54 (m, 4H, β, γ).

[259] This material was dissolved in 1.5 mL dry anisole and stirred with 0.3 mL anhydrous methanesulfonic acid for 3 hours upon which another 0.3 mL anhydrous methanesulfonic acid was added and the solution stirred for 1 hour. The reaction mixture was poured into 6 mL Et_2O and refrigerated for 15 minutes. The heterogeneous biphasic mixture was concentrated to 0.5 mL by rotary evaporation. Twice, an additional 8 mL Et_2O was added and the biphasic mixture centrifuged and the supernatant removed leaving a yellowish gum. This residue was washed twice with 6 mL acetone, centrifuged and the supernatant discarded leaving behind a white-yellow residue. The residue was dissolved in 0.3 mL H_2O and shaken with Amberlite IRA-400. The resin was removed by filtration and washed with 3 mL H_2O. The combined eluent and wash were dried in vacuum yielding a yellow film 0.063 g, (90% yield).

^{1}H NMR (500 MHz, D_2O): δ 4.37 (m, 1H, α), 3.22 (m, 2H, δ) 1.94-1.66 (m, 4H, β, γ); MALDI-MS shows a degree of polymerization varying between six to fourteen residues.

(b) Method 2

[260] To Boc-Arg(Z)$_2$-OH (0.025 g, 0.05 mmol) and H-Arg(Z)$_2$-OH (0.280 g, 0.63 mmol) in 3.0 mL dry DMSO was added diphenylphosphorylazide (219 μl, 1.02 mmol) and triethylamine (236 ul, 1.69 mmol). The reaction was stirred for 48 hours and then poured into 100 mL H_2O. The heterogeneous solution was centrifuged and the precipitate washed 3 x 100 mL H_2O, 3 x 100 mL CH_3OH and 100 Et_2O and then vacuumed dried to obtain 132 mg of solid.

^{1}H NMR (500 MHz, DMSO-d_6): δ 7.31 (m, 10H), 5.21 (m, 1H, benzylic), 5.01 (m, 1H, benzylic), 3.83 (m, 1H, α), 3.34 (m, 2H, δ) 1.54 (m, 4H, β, γ).

[261] The protected polymer was dissolved in 1.5 mL dry anisole and stirred with 1.3 mL anhydrous methanesulfonic acid for 4 hours. The solution was concentrated to 0.5 mL by rotary evaporation. Et_2O (8 mL) was added and the biphasic system centrifuged and the supernatant discarded. Twice, 10 mL acetone was added, the solution centrifuged and the supernatant discarded. The pellet was dried overnight in vacuum and then dissolved in 0.3 mL H_2O and shaken with Amberlite IRA-400. The resin was removed by filtration and washed with 3 mL H_2O. The combined eluent and wash were dried in vacuum yielding a yellow film 0.019, (24% yield).

^{1}H NMR (500 MHz, D_2O): δ 4.37 (m, 1H, α), 3.22 (m, 2H, δ) 1.94-1.66 (m, 4H, β, γ); MALDI-MS shows a degree of polymerization varying between five to eleven residues.

VI Mulitple Attachment of Active Agent to a Peptide

[262] In another preferred embodiment of the invention, more than one active agent may be attached to a peptide. In the case of oligopeptides and polypeptides, the active agents may be distributed randomly or at set intervals along the chain through side-chain attachments, as well as, terminate in either C-capped and/or N-capped active agents. Additionally, in the case of amino acid active agents the amino acid active agent may be interspersed similar to the side-chain

distribution. The distribution may further be grouped intervals of active agents at the ends of the chain or throughout the peptide.

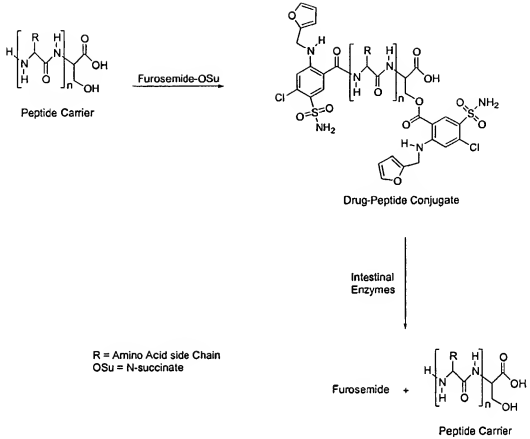
[263] The below example provides a description of the attachment of an active agent to the side chain and C-terminus of a peptide. In the below example, the peptide chain comprises PolyGlu. Additionally, in the below example the active agent is attached through a carboxylate group.

[264] One skilled in the art will appreciate that other peptides can be substituted depending on the active agent selected. Likewise, one skilled in the art will appreciate that the attachment of the active agent can be from different functional groups.

[265] The general scheme for attaching an acitive agent to the N-terminus and the side chain of an amino acid is further illustrated below through the preparation of furosemide conjugated to serine.

VI:A - Preparation of Furosemide Conjugated to Serine

[266] The example below describes the attachment of Furosemide to the side-chain and N-terminus of Polyserine.



[267] To a solution of pSer in NMP was added Furosemide-OSu and N-methyl morpholine (NMM). The reaction was stirred overnight at 70°C. After cooling, reaction was placed in ether and solid was collected by filtration. Solid was suspended in pH 8 water and purified using ultrafiltration. Product was filtered and dried.

[268] The following examples produce an active agent peptide conjugate which results in the attachment of mevastatin, prednisone and pravastatin to the carboxylate side-chain and C-terminus of the peptide. In these examples the amino acid peptide is a polyGlu peptide.

[269] Through the use of protection/deprotection techniques, one skilled in the art would appreciate that the drug could be attached only to the side-chain or only to the C-terminus of the amino acid. Additionally, one skilled in the art would

appreciate that the drug could be attached to a single amino acid at both the C-terminus and side-chain, provided the amino acid had the require functional groups, as with Glu.

VI:B - Preparation of PolyGlu Mevastatin

(i) **AcNGlu$_{15}$(3-mevastatin)$_2$** (SEQ ID NO: 3)

[270] To polyGlu$_{15}$ (SEQ ID NO: 3) (0.116 g, 69 μmol) in 3 mL dry DMF was added 1 mL pyridine and acetic anhydride (20 μl, 207 μmol). After stirring for 21 hours the mixture was acidified with 6 N HCl until pH 1 and then cooled to 4°C. The white precipitate was collected by centrifugation and washed three times with H_2O and then dried under vacuum to yield 11 mg of N-acetylated polyGlu$_{15}$ (SEQ ID NO: 3).

[271] To N-acetylated polyGlu$_{15}$ (SEQ ID NO: 3) (0.011 g, 7 α mol) in 4.8 mL dry DMF was added dicyclohexylcarbodiimide (0.022 g, 108 μmol). After stirring twenty minutes the heterogeneous solution was filtered to remove insoluble dicyclohexylurea and combined with mevastatin (0.042 g, 108 μmol) and N-dimethyl-4-aminopyridine (0.013 g, 108 μmol). The mixture stirred for 23 h whereupon the reaction was quenched by the addition of 20 mL H_2O. The solution was extracted twice with 10 mL $CHCl_3$. The aqueous component was adjusted to pH 3 with 1 N HCl and cooled to 4°C. The resultant white precipitate was isolated by centrifugation and washed three times with 8 mL H_2O. The solid was dissolved in 1 mL H_2O and washed with 1 mL CH_2Cl_2 and twice with 2 mL EtOAc. The aqueous layer was acidified to pH 3 with 1 N HCl, cooled to 4°C, the precipitate isolated by centrifugation and washed twice with 2 mL H_2O. The dried conjugate (2 mg) was shown by ^{1}H NMR to contain fifteen Glu for every two mevastatin molecules.

^{1}H NMR (500 MHz, DMSO-d_6): δ 5.92 (5' mevastatin), 5.72 (3' mevastatin), 5.19 (4' mevastatin), 5.17 (8' mevastatin), 5.12 (3 mevastatin), 4.41 (5 mevastatin), 4.03 (α, Glu), 2.25 (γ, Glu), 1.88 (β, Glu), 0.82 (4",2' allylic methyl mevastatin), 1.17 (2" mevastatin).

(ii) **Glu_{15}(3-mevastatin) (160)** (SEQ ID NO: 3)

[272] To Glu_{15} (SEQ ID NO: 3) (0.151 g, 77 μmol) in 3 mL dry DMF was added dicyclohexylcarbodiimide (0.239 g, 1.159 mmol) and the reaction stirred for 4 hours under argon. The white precipitate was removed and N-dimethyl-4-aminopyridine (0.141 g, 1.159 mmol) and mevastatin (0.222 g, 0.569 mmol) were added dissolved in 10 mL $CHCl_3$. The reaction stirred for 21 hours under argon whereupon the precipitate was removed. The solution was concentrated by rotary evaporation and added to 40 mL saturated NaCl (aq) adjusted to pH 8. The homogeneous solution was extracted three times with 20 mL $CHCl_3$ and then ultrafiltered (Amicon regenerated cellulose, YM1, NMWL 1,000). The retentate was dried in vacuum to yield 8 mg of a white residue which showed a ratio of 15 Glutamic acids to one mevastatin by ^1H NMR.

^1H NMR (500MHz, D_2O): δ 5.92 (5' mevastatin), 5.72 (3' mevastatin), 5.19 (4' mevastatin), 5.17 (8' mevastatin), 5.12 (3 mevastatin), 4.41 (5 mevastatin), 4.03 (α, Glu), 2.25 (γ, Glu), 1.88 (β, Glu), 0.82 (4",2' allylic methyl mevastatin), 1.17 (2" mevastatin).

(iii) **BocGlu(3-mevastatin)O-tBu**

[273] To BocGlu(OSu)O-tBu (0.181 g, 453 μmol) and mevastatin (0.177 g, 453 μmol) in 40 mL $CHCl_3$ was added N-dimethyl-4-aminopyridine (0.055 g, 453 μmol). The reaction was heated to reflux for 7 hours under argon and then allowed to stir at 20° C for 8 hours. The solvent was removed by rotary evaporation and the residue purified by flash chromatography (8:1-1:1 hexane:EtOAc) to provide the conjugate as a clear film (0.038 g, 11%).

R_f (3:1 hexane:EtOAc): 0.22; ^1H NMR (500 MHz, $CDCl_3$): δ 5.97 (d, 1H, 5'), 5.73 (dd, 1H, 3'), 5.55 (s, 1H, 4'), 5.32 (s, 1H, 8'), 5.24 (dd, 1H, 3), 5.09 (d, 1H, NH), 4.48 (m, 1H, 5), 4.20 (m, 1H, α), 2.78 (m, 2H, 2), 2.37 (m. 4H, 2', 2", γ), 1.45 (s, 18H, t-Bu), 1.12 (d, 3H, 2"-CH_3), 0.88 (m, 6H, 4", 2'-CH_3).

VI:C – Preparation of PolyGlu Prednisone

[274] To Glu_{15} (SEQ ID NO: 3) (0.350 g) in 30 mL of DMF was added bromo-tris-pyrrolindinophosphonium hexafluorophosphate (0.510 g, 1.1 mmol) and

N-methylmorpholine (1 mL, 9.3 mmol). The mixture was stirred for 30 min under Ar whereupon N-dimethyl-4-aminopyridine (0.067 g, 0.5 mmol) and prednisone (0.489 g, 1.4 mmol) were added. After stirring for 14 h, the solvent was removed under reduced pressure. The residue was dissolved in 50 mL H_2O and acidified with 6 N HCl to pH 3. The precipitate was filtered and washed with 30 mL $CHCl_3$. The solid was then dissolved in 70 mL H2O pH 8 and extracted twice with 40 mL $CHCl_3$. The aqueous layer was ultrafiltered (Amicon regenerated cellulose, YM1, NMWL 1,000) with 150 mL H_2O and the retentate dried in vacuum to provide a white residue. ^1H NMR (DMSO) analysis indicated 4:1 Glu:Prednisone ratio.

VI:D – Preparation of Glu_{15}(Gly-Pravastatin) (SEQ ID NO: 3)

[275] This example describes the C-terminus attachment of an alcohol to a single amino acid (glycine) with subsequent attachment to the C-terminus and side-chain of polyglutamic acid.

[276] To N-Boc-Glycine (0.247 g, 1.41 mmol) in 5 mL dry DMF was added dicyclohexylcarbodiimide (0.138 g, 0.67 mmol). After stirring for 1 h under Ar the solution was filtered through glass wool to remove insoluble urea. Pravastatin sodium (0.300 g, 0.67 mmol) was added followed by N-methylmorpholine (147 µl, 1.34 mmol). The mixture was stirred for an additional 23 hours under argon whereupon the solution was filtered through glass wool and the solvent removed by rotary evaporation. The residue was purified by flash chromatography (30:1–8:1 $CHCl_3$:CH_3OH) to provide the peracylated statin as a white solid (0.118 g).

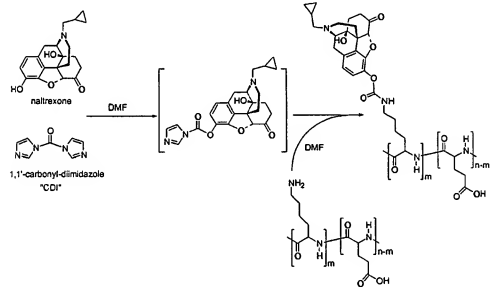
R_f = 0.23 (6:1 $CHCl_3$:CH_3OH); ^1H NMR (500 MHz, $CDCl_3$): δ 5.97 (m, 1H, 5'), 5.89 (m, 1H, 3'), 5.58 (bs, 1H, 6'), 5.40 (bs, 1H, 4'), 5.16 (bs, 1H, 8'), 3.92 (m, 6H, α), 2.69-2.34 (4H, 2, 2, 2', 2"), 1.43 (s, 18H, t-Bu), 1.09 (d, 3H), 0.87 (m, 6H).

VII General Description of Linker for Attachment to Peptide

[277] For those drugs which are not amenable to attachment through the C-terminus, N-terminus or side-chain, a linker is required for a stable covalent

attachment. For example, direct covalent attachment of an amino acid to a ketone would not produce a stable entity. However, the insertion of a carbonyl between the active agent and the carrier peptide should provide enhanced stability of the Active Agent/Peptide Conjugate. Furthermore, the formation of a ketal using a reagent with the appropriate functionality in the agent provides a link between the active agent and the amino acid conjugate with the desired stability. Another example of this type of linker is the DHP linker. Ideally, these linkers should be removable *in vivo*. From the below examples one skilled in the art would appreciate how to derive other linkers and attachment functionalities.

VII:A - Preparation of Carbamate linked Naltrexone-polymer conjugates



[278] Naltrexone-hydrochloride (520 mg, 1.37 mmol) and 1-1'-carbonyl-diimidazole (CDI) (202 mg, 1.25 mmol) were dissolved in anhydrous DMF (5 mL). The reaction was then allowed to stir for 1 hour at room temperature under argon. Glutamic acid-lysine copolymer (Glu_nLys_m , 2.5 mmol of free lysine sidechains*) was then added as a suspension in 15 mL DMF, and the reaction allowed to continue stirring under argon at room temperature for 2 days. The solvent, DMF, was then

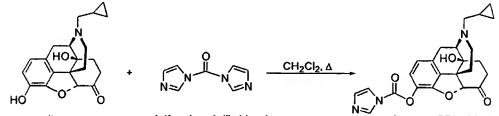
removed by rotary evaporation under high vacuum, leaving a green solid. The solid was dissolved in water (20 mL), and the aqueous solution filtered/concentrated using ultrafiltration (1000 mw cutoff) to remove small molecular weight starting materials and byproducts. Two aliquots of water (10 mL each) were added and the solution filtered/concentrated after each addition to a final volume of ~2 mL. The remaining solution was freed of solvent by rotary evaporation and the resulting solid dried over night in a vacuum chamber at room temperature. This afforded the carbamate conjugate (642 mg, 43% yield assuming saturation of available lysine sidechains) with an approximate loading of 1:4 (naltrexone/amino acid residue) as estimated by ^{1}H-NMR.

^{1}H NMR (360 MHz, DMSO-d_6): δ 6.78 and 6.61 (bs, 1H each, naltrexone-aromatic); 2.74 (bs, ~8H, γ-Glu); 2.20 (bs, ~8H, β-Glu), 0.50 (bs, 2H, naltrexone-cyclopropyl) and 0.16 (bs, 2H, naltrexone-cyclopropyl).

(* mmol of Lysine sidechains is estimated based on a 1:1 Glu/Lys ratio as previously determined by NMR. This copolymer was prepared from Lys(Boc)-NCA and Glu(OtBu)-NCA using standard NCA polymerization methods. The resulting polymer (1.00 g, 2.5 mmol Lys) was deprotected using 4N HCl in Dioxane)

VII:B - Preparation of Carbonate linked Naltrexone-polymer conjugates:

(i) Reaction of Naltrexone (free base) with CDI

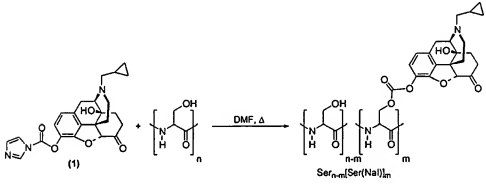


[279] CDI (0.522 g, 3.2 mmol) was dissolved at room temperature in 20 mL of dry methylene chloride in a flask charged with argon. The naltrexone (1.00 g, 2.9 mmol) dissolved in mehtylene chloride (20 mL) was then added drop wise to the CDI solution. An additional 10 mL of methylene chloride was used to rinse the

vessel that had contained the naltrexone, and the wash added to the reaction mixture. The reaction was heated to 50°C, and allowed to stir over night under argon at a temperature between 40 and 50°C. The solvent was then removed by rotary evaporation under high vacuum. ^{1}H-NMR indicated that the tacky solid contained a mixture of imidazole, the adduct **1** and unreacted starting materials. Imidazole and compound **1** were the dominant components.

^{1}H NMR (360 MHz, d_6-DMSO): δ 8.27 (bm, 1H, **1**); 7.74 (bs, 2 H, imidazole); 7.53 (t, 1H, **1**); 7.24 (bs, 1H, imidazole); 7.14 (bm, 1H, **1**); 6.95 (d, 1H, **1**) and 6.73 (d, 1H, **1**).

(ii) **Reaction of Naltrexone-CDI adduct with Ser_n**

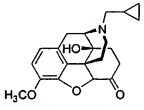


[280] The solid from step 1 was dissolved in anhydrous N-methylpyrrolidinone (NMP), and solid Ser_n (0.51 g, 5.9 mmol) added to the solution. The reaction mixture was then heated to 60 °C under argon, and allowed to stir under argon, over night at a temperature between 50 and 60°C. The organic solution was then diluted into 100 mL of water. Precipitate formed immediately, and the solid (*A*) was collected by centrifuge, and the pellets then dried over night in a vacuum chamber. The water in the supernatant was removed by rotary evaporation, and the NMP solution that remained was diluted into ether (100 mL). Again, precipitate formed immediately. This solid (*B*) was collected by filtration and then dried over night in a vacuum chamber. Both solids were hygroscopic and appeared similar in composition by TLC (3:1 $CHCl_3/CH_3OH$). Therefore, solids *A* and *B* were combined and dissolved/suspended in ~50 mL water. Ultrafiltration (1000 mw

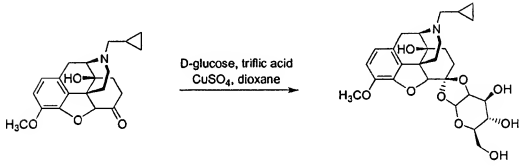
cutoff) was used to remove impurities such as unreacted naltrexone and imidazole, leaving the Ser_n and the naltrexone conjugate, Ser_{n-m} $[Ser(Nal)]_m$. The suspended material was washed with 5 aliquots of water (10 mL each), and then pelletted by centrifugation. The polymer conjugate was then dried over night in a vacuum chamber. This afforded 80 mg (~5% yield) of material with an estimated loading of 1:19 naltrexone/serine (based on ^{1}H-NMR).

^{1}H NMR (360 MHz, DMSO-d_6): δ 5.03 (bs, ~19H, α-Ser); 0.59 (bs, 2H, naltrexone-cyclopropyl) and 0.34 (bs, 2H, naltrexone-cyclopropyl).

VII:C – Preparation of Methyl Naltrexone – Glucose Ketal Conjugate



Methyl Naltrexone



(i) Preparation of 3-Methyl Naltrexone

[281] Naltrexone (6.0 g, 16.5 mmol) was dissolved in 100 mL distilled water. The solution was titrated with 1N NaOH to a final pH of 11.8. In the course of the titration, neutral naltrexone precipitated from solution and then went back into solution. Upon reaching pH 11.8, the solvent was removed by rotary-evaporation under high vacuum, and the resulting solid stored under vacuum over night at room temperature. The solid was then suspended/dissolved in anhydrous tetrahydrofuran

(200 mL) and allowed to stir at room temperature under argon. A solution of iodomethane (2.1 mg, 33 mmol) in 50 mL of tetrahydrofuran was added dropwise over the course 30 minutes. The reaction was then allowed to stir an additional 3 hours at room temperature under argon. The solvent was then removed by rotary-evaporation under reduced pressure. The residual solid was then dissolved in 40 mL of $CHCl_3$ and the organic solution washed with 30 mL of saturated NaCl, 3 x 30 mL of 1N NaOH and finally twice more with 30 mL saturated aqueous NaCl. The organic solution was collected and dried over sodium sulfate. Removal of solvent by rotary-evaporation and drying over night under vacuum afforded pure 3-methylnaltrexone (5.6 g, 15.8 mmol, 96% yield) as a brown residue and composition determined by TLC and ^{1}H-NMR.

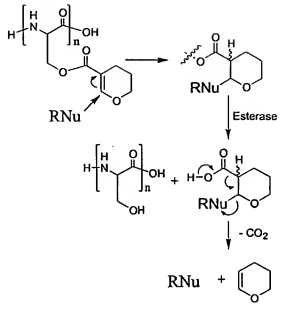
^{1}H NMR (360 MHz, $CDCl_3$): δ 6.677 (d, 1H, naltrexone aromatic), 6.591 (d, 1H, naltrexone aromatic), 3.874 (s, 3H, methoxy group.), 0.6-0.5 (m, 2H, naltrexone cyclopropyl) and 0.2-0.1 (m, 2H, naltrexone cyclopropyl).

(ii) **Preparation of Methyl Naltrexone – Glucose Ketal Conjugate**

[282] To a solution of methyl naltrexone (0.200 g, 0.56 mmol) in dioxane (20 mL) was added D-α-glucose (2.02 g, 11.2 mmol), triflic acid (0.05 mL, 0.62 mmol), and $CuSO_4$ (1.00 g). The reaction mixture was stirred at ambient temperatures for 4 days. Reaction was then filtered, neutralized with saturated $NaHCO_3$ and filtered again. Dioxane and water were removed and the residue was taken up in $CHCl_3$ and extracted with water (3 x 100mL). The organic layer was dried over $MgSO_4$ and solvents were removed under reduced pressure. Crude product was purified over silica gel (0-10% MeOH in $CHCl_3$) to obtain the ketal conjugate (0.010 g) in a 1:1 mixture with free methyl naltrexone.

^{1}H NMR (360 MHz, $CDCl_3$): δ 0.14 (br s, 4H, naltrexone cyclopropyl), 0.53 (br m, 4H, naltrexone cyclopropyl), 0.90 (m, 2H, naltrexone cyclopropyl), 1.48 (m, 6H, naltrexone), 2.19-2.78 (m, 12H, naltrexone), 3.03 (m, 2H, naltrexone), 3.75 (q, 2H, glucose), 3.87 (m, 8H, naltrexone CH_3 and glucose), 3.97 (q, 2H, glucose), 4.14 (q, 1H. glucose), 4.33 (t, 1H, glucose), 4.66 (s, 1H, naltrexone), 6.65 (m, 4H, naltrexone).

VII:D - DHP Linker Chemistry



[283] All reagents were used as received. ^{1}H NMR was run on a JEOL 500 MHz (500) NMR spectrophotometer using tetramethylsilane as an internal standard. Thin layer chromatography was performed using plates precoated sith silica gel 60 F_{254}. Flash chromatography was performed using silica gel 60 (230-400 mesh).

(i) Method (a)

[284] To 3,4-dihydro-2H-pyran-5-carboxylic acid [M. Hojo, R. Masuda, S. Sakaguchi, M. Takagawa. 1986, A Convenient Synthetic Method for B-Alkoxy- and B-Phenoxyacrylic Acids and 3,4-Dihydro-2H-pyran-5- and 2,3-Dihydrofuran-4-carboxylic Acids. Synthesis, 1016-17.] (0.044 g, 343 μmol) in 2 mL $CHCl_3$ was added phosphorous pentachloride (0.143 g, 687 μmol). After stirring overnight Fmoc-Ser (0.101 g, 309 μmol) was added. After stirring for an additional 72 hours, 5 mL CH_2Cl_2 was added and the mixture washed with 5 mL saturated NaCl. The solvent was removed by rotary evaporation and the residue purified by flash

chromatography (15:1:0-10:1:0-100:10:1 $CHCl_3$:MeOH:HOAc) to provide the target as a white solid (23%).

R_f = 0.23 (6:1 $CHCl_3$:MeOH); ^1H NMR (360 MHz, $CDCl_3$): δ 7.81-7.25 (m, 9H, aromatics and vinyl), 5.63 (s, 1H, carbamate), 4.64 (t, 1H, a), 4.47 (m, 2H, serine CH_2), 4.23 (m, 3H, Fmoc CH_2 and CH), 4.04 (m, 2H, DHP's OCH_2), 2.24 (m, 2H, allylic), 1.86 (m, 2H, homoallylic).

(ii) Method (b)

[285] To 3,4-dihydro-2H-pyran-5-carboxylic acid (0.527 g, 4.1 mmol) in 30 mL $CHCl_3$ was added diisopropylcarbodiimide (0.260 g, 2.1 μmol). After stirring 2hours, Fmoc-Ser (0.673 g, 2.1 mmol) and N-dimethyl-4-aminopyridine (0.502 g, 4.1 mmol) was added and the reaction stirred an additional 14h. More diisopropylcarbodiimide (0.260 g) was added and the reaction mixed for an additional 24 h whereupon an additional diisopropylcarbodiimide (0.130 g) was added and the reaction stirred 24 more hours. The solvent was removed by rotary evaporation and the residue repeatedly purified by flash chromatography to provide the target as a white solid (7%).

VIII *In Vitro* Performance Studies of Various Active Agents and Amino Acids Conjugates

VIII:A – Materials and Methods of the *In Vitro* Performance Studies for Peptide Conjugated Active Agents Testing

[286] Esterase (EC 3.1.1.1; from porcine liver), lipase (EC 3.1.1.3; from porcine pancreas), amidase (EC 3.5.1.4; from *Pseudomonas aeruginosa*), protease (EC 3.4.24.31; type XIV, bacterial from *Streptomyces griseus*; also known as pronase), pancreatin (EC 232-468-9; from porcine pancreas), pepsin (EC 3.4.23.1; from porcine stomach mucosa), tris-HCl, methimazole were all purchased from Sigma. Buffers used in the digestive assays were prepared as follows: reducing buffer [110mM sodium chloride, NaCl, 50mM methimazole, 40mM tris-HCl, adjust pH to 8.4 with 1N sodium hydroxide, NaOH], Intestinal Simulator (IS) buffer [100mM monobasic potassium phosphate, adjust pH to 7.5 with 1N NaOH], Gastric

Simulator (GS) buffer [69mM NaCl, adjust pH to 1.2 with HCl], esterase buffer [10mM borate buffer pH to 8 with NaOH], lipase and amidase buffer [100mM monobasic potassium phosphate pH to 7.5 with NaOH].

[287] The proteolytic release of the active agent from the peptide conjugates was determined in different assays. Peptide conjugates were shaken at 37°C in the presence of pronase, pancreatin, esterase, lipase, or amidase for 24 hours or pepsin for 4 hours. Stock solutions of each conjugate (0.5-2.0 mg/mL) and enzymes (protease, reducing buffer, 6mg/mL; pancreatin, IS buffer, 20 mg/mL; pepsin, GS buffer, 6.40 mg/mL; esterase, esterase buffer, 1.02 mg/mL; lipase, lipase buffer, 0.10 mg/mL; amidase, amidase buffer, 0.10 µl/mL) were prepared. For protease, pancreatin, and pepsin digestion, conjugate and enzyme were diluted 2-fold in the assay in a final volume of 2 mL. After the indicated incubation time for each assay, 2 mL of acetonitrile, MeCN, containing 1% of phosphoric acid, H_3PO_4, was added to each sample to stop digestion, and samples were centrifuged to remove gross particulate matter. Any remaining particulate was filtered with a 0.2 µm nylon syringe filter (Whatman) prior to HPLC analysis.

[288] Enzyme digested conjugates were analyzed for the presence of unconjugated active agent by reversed phase HPLC (C18, 4.6 x 250mm, 5µm, 300A) using the following conditions: mobile phase – Lotus buffer (4.5 mL of H_3PO_4, 8.8 mL triethylamine, pH = 3.5)/ THF/ MeCN [68.6/4.5/26.9] or TBA-phosphate buffer (10mM tetrabutyl ammonium chloride, 10mM monobasic sodium phosphate, pH = 6.0)/ MeCN [65/35]; injection volume – 20 µl; flow rate – 1 mL/min; UV – 230 nm. Retention times of active agent were determined from standards in a calibration curve which was used to calculate the concentrations of enzymatically released active agent.

VIII:B – *In Vitro* Performance Studies Results

[289] The Table 8 below depicts active agent conjugates that were tested with the stomach, inestine, and pronase simulator.

- Table 8: *In vitro* Test Results of Simulator -

Generic Name	Peptide	Stomach	Intestine	Pronase
Atenolol	Glu	2%	63%	0%
Lisinopril	Glu	0.83%	13.75%	25.80%
Metoclopramide	Glu	0%	95.20%	6.50%
Acyclovir	Glu	16%	5%	10%
Gemfibrozil	Lys	0%	3.60%	2%
Levo/Carbidopa	Glu	0.70%	6.84%	2.89%
Quetiapine	Glu	1%	12.80%	13.70%
Sertraline	Glu	32.50%	0%	0%
Cephalexin	Glu	3%	100%	53%
Ciprofloxacin	Glu	2.50%	1%	0.53%
Mesalamine	Glu	11%	10%	46%
Metronidazole	Glu	20.20%	64.30%	28.90%
Stavudine**	Glu	29.70%	20.60%	88.60%
Zalcitabine	Glu	0%	2.50%	29.10%
Acetaminophen	Glu	0%	1.2%	0.2%
Naproxen	Lys	0%	0%	0%
Fexofenadine	Glu	0%	5%	25%
Furosemide	Ser	0%	0%	0%
Ibuprofen	Lys	4.2%	91%	40%

VIII:C - Caco-2 Human Intestinal Epithelial Cells Studies

[290] Monolayers of Caco-2 human intestinal epithelial cells are increasingly being used to predict the absorption of orally delivered drugs. The Caco-2 transwell system and other *in vitro* assays were used to evaluate the performance of Polythroid. Findings indicated that Polythroid enhance oral delivery of thyroid hormones for the treatment of hypothyroid disorders.

(i) **Caco-2 human intestinal epithelial cell assay**

[291] Caco-2 cells were grown on the surface of collagen coated wells in a 24 well format to form confluent monolayers that represent small segments of the intestine. The wells were removable and contain a top chamber representing the apical side (facing the lumen of the intestine) and a bottom chamber representing the basolateral side (site of serosal drug absorption). The integrity of the epithelial barrier was monitored by testing the electrical resistance across the monolayer.

Absorption of drugs was studied by adding sample to the apical side and assaying the concentration of the drug in the basolateral chamber following incubation.

(ii) Intestinal epithelial cell proteases digest Polythroid

[292] Polythroid is a synthetic polymer of glutamic acid with T4 and T3 covalently attached by a peptide bond linkage. The polymer is the delivery vehicle for the thyroid hormones and is not designed to cross the intestinal barrier itself. Rather, it was designed to release T4 and T3 in a time dependent manner. Release of the thyroid hormones is dependent on the enzymatic cleavage of the glutamic acid polymer. In theory, this will result from Polythroid encountering proteolytic enzymes as it descends the intestinal tract. Proteins are digested into small peptides by gastric pepsin and pancreatic enzymes secreted into the small intestine. Intestinal epithelial cells then function to further breakdown the small peptides. They accomplish this with proteolytic enzymes referred to as brush border proteases that are attached to the cell surface.

[293] Monitoring the effect of brush border peptidases on Polythroid required development of an assay to specifically distinguish Polythroid from polyglutamic acid and the thyroid hormones. Therefore, we developed an enzyme-linked immunosorbent assay (ELISA) that specifically recognizes Polythroid. The assay employs antibodies against the glutamic acid polymer to capture Polythroid and antibodies to T4 or T3 to detect the presence of Polythroid. The assay has no cross-reactivity with polyglutamic acid or the thyroid hormones themselves. Consequently, proteolytic degradation of Polythroid results in T4 and T3 release from the polymer and a corresponding decrease in ELISA reactivity. The Polythroid specific ELISA can, therefore, be used to monitor the breakdown of Polythroid.

[294] The Polythroid specific assay was used to analyze *in situ* digestion of Polythroid in Caco-2 cell cultures. Different concentrations of Polythroid were added to the apical side of Caco-2 cells and incubated for 4 hours in PBS at 37°C (n = 4). The apical side Polythroid concentration was measured by Polythroid specific ELISA before and after the 4 hour incubation (Figure 2). At the relatively high

concentration of 100 micrograms, 26% of Polythroid was degraded, whereas at a 10-fold lower concentration 84% of the Polythroid was degraded. When a concentration of 0.5 micrograms was added (closer to the concentrations that would be encountered by the intestine in a normal human dose) the amount of Polythroid remaining after 4 hours of incubation was below the limit of detection for the ELISA (10 ng) indicating essentially complete digestion. The loss of Polymer in the apical chamber was not due to absorption of Polythroid across the monolayer since the basolateral chamber contained no detectable Polythroid in any of the experiments (see below). We cannot rule out cellular uptake of Polythroid, however, enzymatic digestion is likely to account for most, if not all, of the decrease in Polythroid concentration on the apical side. At the higher concentrations, it would be difficult for cellular uptake to account for such a large difference in the remaining Polythroid.

(iii) PolyT4 enhances absorption of T4 across Caco-2 monolayers

[295] Absorption of T4 was monitored in the Caco-2 transwell system (n = 4). PolyT4 (10 micrograms) was added to the apical side of the transwells. T4 was added to the apical side at a concentration equal to the T4 content of PolyT4. A commercial ELISA for T4 was used to determine the level of T4 in the basolateral chamber following incubation for 4 hours at 37°C (Figure 3). A significantly higher amount of T4 was absorbed from PolyT4 as compared to CaCo-2 cells incubated with the amount of T4 equivalent to that contained in the polymer.

(iv) Polythroid Does Not Cross Caco-2 Monolayers

[296] In order to determine if Polythroid itself crosses the Caco-2 monolayer we used the Polythroid specific ELISA to measure the amount of polymer in the basolateral chamber after incubation with Polythroid at a high concentration (100 micrograms). After 4 hours incubation, samples (n = 4) from the basolateral side showed no reactivity in the ELISA (Figure 4). The limit of detection for Polythroid is 10 ng, therefore, less than 1/10,000 of the Polythroid was absorbed. In conclusion, within the limits of ELISA detection, Polythroid does not cross the Caco-2 monolayer.

(v) **Conclusions and Summary**

[297] The following discussion recites *in vitro* performance studies conducted with regard to specific embodiments of the present invention. Although these performance studies describe specific embodiments of the present invention, it is not limited to these embodiments. Alternative embodiments and modifications which would still be encompassed by the invention may be made by those skilled in the art, particularly in light of the foregoing teachings. Therefore, this invention is intended to cover any alternative embodiments, modifications or equivalents which may be within the spirit and scope of the invention.

[298] The *in vitro* performance assays provide the following conclusions. Active agents are released from peptide conjugates by pancreatic and intestinal cell proteases. T4 and T3 released from Polythroid are absorbed across intestinal monolayers. PolyT4 enhances absorption of T4 across intestinal epithelium *in vitro*. Polythroid itself does not cross the intestinal epithelial barrier *in vitro*. The kinetics of time release may be controlled by the method of Polythroid synthesis.

[299] Data from the *in vitro* intestinal epithelial model suggests that attachment of T4 to polymers of glutamic acid may enhance absorption of the thyroid hormones, perhaps by providing a second mechanism of uptake and/or enhancing solubility of the hormones. Polythroid itself does not cross the intestinal epithelial barrier in the *in vitro* Caco-2 model. Thus, any concerns about systemic effects of the polymer are minimized since it should not be absorbed into the bloodstream.

IX: *In Vivo* Performance Studies of Various Active Agents and Amino Acids Conjugates

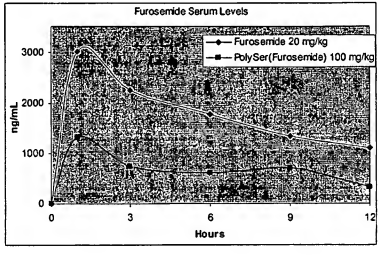
IX:A - *In Vivo* Performance Studies of Polymer-Drug Conjugates

[300] Pharmacokinetics of various parent drugs and amino acid polymer drug conjugates containing an equivalent dose were tested *in vivo* by oral gavage of female Sprague Dawley rats. Doses (mg/kg) were given as solutions in water or

sodium bicarbonate buffer. Serum was collected under anesthesia by jugular venipuncture for the first bleed and cardiac puncture for the second bleed. Collections were taken from 5 animals/set predose (jugular) and at 6 hours (cardiac); 1 hour (jugular) and 9 hours (cardiac); and 3 hours (jugular) and 12 hours (cardiac). Serum drug levels were determined by LC/MS/MS or ELISA.

IX:B - In Vivo Performances of Furosemide (Side-chain)

Study 6856-120	0	1 hour	3 hours	6 hours	9 hours	12 hours
Furosemide	3	3017.4	2250.0	1771.5	1345.8	1112.6
PolySer(Furosemide)	5.4	1320.1	738.6	608.1	706	333.2

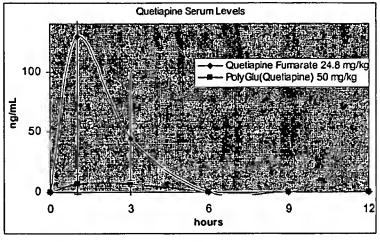


[301] Study 6856-120 shows the serum concentration levels of Furosemide vs. PolySer (Furosemide) conjugate containing an equivalent amount of Furosemide. Furosemide had an (AUC) of 21,174 as compared to the conjugate which had an AUC of 8,269 (39.1 % relative to the parent drug). The figure above shows the serum concentration curves of the parent drug vs. PolySer (Furosemide) conjugate. The 9 hour serum level of the PolySer(Furosemide) conjugate was 95.5% of its 3 hour level, whereas the 9 hour serum level of the parent drug was only 59.8% of its 3 hour level. This and the relative flatness of the PolySer (Furosemide) conjugate serum concentration curve between 3 and 9 hours, as compared to that of the curve

for the parent drug, illustrate sustained release by the PolySer (Furosemide) conjugate.

IX:C - In Vivo Performances of Quetiapine

Study 6856-117	0	1 hour	3 hours	6 hours	9 hours	12 hours
Quetiapine Fumarate	0	129.4	44.6	0	0	0
PolyGlu(Quetiapine)	0	6.8	7.16	0	0	0



The results of study 6856-117 are shown in the table and the figure above.

IX:D – Summary of *In Vivo* Performances Studies of Various Polymer-Drug Conjugates

- Table 9. Relative Percent of Various Amino Acid Polymer Drug Conjugates AUCs vs. Parent Drug AUCs -

Generic Name	Peptide	Rat
Atenolol	Glu	38.5% & 42.6%
Furosemide	Glu, Ser	2.1%(E),39.1%(S)
Lisinopril	Glu	ND
Metoclopramide	Glu	ND
Acyclovir	Glu	ND
Quetiapine	Glu	9%
Naltrexone	E,K,S,ES,EW	14%(E),9.4%(K)
Ibuprofen	Lys	0%

[302] The relative percent of area under the serum concentration curves (AUCs) of various amino acid polymer drug conjugates compared to parent drug AUCs is shown in Table 9. The relative percents ranged from 9.4% to 42.6% and varied depending on the amino acid content of the polymer drug conjugate. These examples illustrate the ability to covalently bond various drugs to polymers of various amino acids and affect release and absorption of the bound drug into the sera when given as an oral dose to an animal.

IX *In vitro* and *In* vivo performance of Polyserine-Naltrexone conjugate (carbonate linked)

X:A - *In Vivo* performance of Polyserine-Naltrexone conjugate (rat model)
(Lot no. BB-272, 1:6 naltrexone:serine ratio)

[303] Polyserine-naltrexone conjugates were tested in male Sprague Dawley rats (~ 250 g). Defined doses were delivered orally in gelatin capsules containing purified dry powder polyserine-naltrexone conjugates or naltrexone. No excipients were added to the capsules.

[304] Content of naltrexone in the PolySerine-Naltrexone conjugate was estimated to be 30% as based on the 1:6 ratio of naltrexone:serine determined by NMR. Polyserine-naltrexone conjugate was given to four rats at a dose of 12 mg which contained 3.6 mg of naltrexone. Doses of naltrexone (3.6 mg) equivalent to the naltrexone content of the conjugate were also given to four rats. Capsules were delivered orally to rats at time-zero using a capsule dosing syringe. Serum was collected from rats 2, 4, 6, 9, and 12 hours after capsule delivery. Serum naltrexone concentrations were determined by ELISA using a commercially available kit (Nalbuphine, product #102819, Neogen Corporation, Lansing MI).

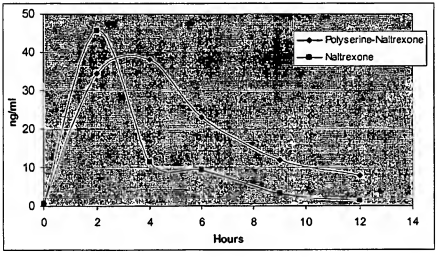
Table 10. Serum Concentrations (ng/mL) of Individual Rats Fed; PolySerine-Naltrexone Conjugate vs. Naltrexone -

	Polyserine-naltrexone				Naltrexone			
Hours	Rat #1	Rat #2	Rat #3	Rat #4	Rat #1	Rat #2	Rat #3	Rat #4
2	58	35	22	22	33	91	37	22
4	66	46	14	27	6	25	12	3
6	34	21	11	26	13	10	8	6
9	22	13	4	10	3	6	2	1
12	8	16	3	5	1	2	1	2

- Table 11. Mean Serum Concentrations of PolySerine-Naltrexone vs. Naltrexone -

Hours	Polyserine-naltrexone (ng/ml +/- SD)	Naltrexone (ng/ml +/- SD)
2	34 +/- 17	46 +/- 31
4	38 +/- 23	11 +/- 10
6	23 +/- 10	9 +/- 3
9	12 +/- 8	3 +/- 2
12	8 +/- 6	1 +/- 1

- Serum Concemtration Curve of PolySerine-Naltrexone vs. Naltrexone -



[305] Serum levels of individual animals are shown in Table 10. Mean serum levels are shown in Table 11. As shown in the figure above, serum levels spiked earlier for naltrexone (2 hours) than for the drug administered as a polyserine-naltrexone conjugate (4 hours). Serum levels of naltrexone for the polyserine-naltrexone conjugate remained elevated considerably longer than for naltrexone. Additionally, the peak level was significantly lower for the polyserine-naltrexone conjugate. It should be noted that the 2 hour time point was the first measurement of naltrexone serum levels. Since this was the peak level measured for naltexone it can not be determined whether or not levels peaked at a higher concentration earlier. Consequently, it was not possible to accurately determine the

Cmax or area under serum concentration curve (AUC) for naltrexone in this experiment.

X:B - *In Vivo* performance of PolySerine-Naltrexone conjugate

(Lot no. BB-301, 1:10 naltrexone:serine ratio)

[306] Polyserine-naltrexone conjugates were tested in Sprague-dawley rats (~ 250 g). Defined doses were delivered orally in gelatin capsules containing purified dry powder polyserine-naltrexone conjugates or naltrexone. No excipients were added to the capsules.

[307] Content of naltrexone in the polyserine-naltrexone conjugate BB-272 was estimated to be 30% as based on the 1:6 ratio of naltrexone:serine determined by NMR. Polyserine-naltrexone conjugate was given to five rats at a dose of 12.9 mg which contained 3.6 mg of naltrexone. Doses equivalent to the naltrexone contained in the batch of polyserine-naltrexone (BB-301) were also given to five rats. Additionally, half the equivalent dose (1.8 mg) was given at time-zero, followed by a second half-dose at 6.5 hours to five rats.

[308] Capsules were delivered orally to rats at time-zero using a capsule delivery syringe. Serum was collected at 0.5, 1.5, 3, 5, 8, 12, 15 and 24 hours after capsule delivery for the polyserine-naltrexone (BB-301) and equivalent naltrexone dosed rats. Serum was collected at 0.5, 1.5, 3, 5, 8, 11.5, 14.5 and 24 hours after capsule delivery for rats dosed with half-equivalent doses at 0 and 6.5 hours. Serum naltrexone concentrations were determined by ELISA using a commercially available kit (Nalbuphine, product #102819, Neogen Corporation, Lansing MI).

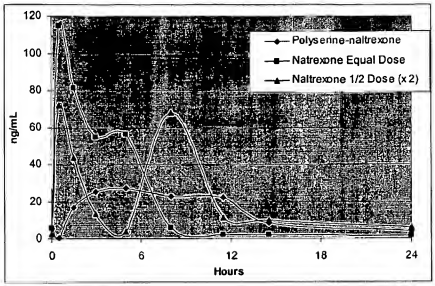
- Table 12. Serum Concentrations (ng/mL) of Individual Rats Fed; PolySerine-Naltrexone Conjugate vs. Naltrexone -

	Polyserine-naltrexone					Naltrexone (equal dose)				
Hours	Rat #1	Rat #2	Rat #3	Rat #4	Rat #5	Rat #1	Rat #2	Rat #3	Rat #4	Rat #5
0.5	0	0	0	1	0	141	128	126	142	39
1.5	5	4	12	38	23	85	79	46	95	102
3	21	12	24	16	52	62	44	30	46	91
5	20	17	23	38	37	193	16	8	19	45
8	22	14	32	32	13	6	2	5	4	19
12	10	47	29	19	7	1	2	3	2	3
15	8	7	13	9	5	1	1	2	2	4
24	4	4	4	4	3	1	1	3	2	2

- Table 13. Mean Serum Concentrations of PolySerine-Naltrexone vs. Naltrexone (equal dose) vs. Naltrexone (1/2 dose x 2) -

Hours	Polyserine-naltrexone (ng/ml +/- SD)	Naltrexone (equal) (ng/ml +/- SD)	Naltrexone (1/2 X2) (ng/ml +/- SD)
0.5	0	115 +/- 47	72 +/- 69
1.5	17 +/- 14	82 +/- 25	44 +/- 46
3	25 +/- 16	55 +/-26	13 +/- 11
5	27 +/- 10	56 +/- 16	4 +/- 3
8	23 +/- 9	7 +/- 8	68 +/- 32
11.5	NA	NA	11 +/- 9
12	22 +/- 16	2 +/- 1	NA
14.5	NA	NA	10 +/- 3
15	8 +/- 3	2 +/- 1	NA
24	4 +/- 0.4	2 +/- 1	6 +/- 1

- Serum concentration curves of PolySerine-Naltrexone vs. Naltrexone (equal dose) vs. Naltrexone (1/2 dose, x 2) -



[309] Serum levels of individual animals are shown in Table 12. Mean serum levels are shown in Table 13. As shown in the figure above, naltrexone serum levels spiked earlier (0.5 hours) for naltrexone than for the drug administed as a polyserine-naltexone conjugate (5 hours). Serum levels of naltrexone for the polyserine-naltrexone conjugate remained elevated considerably longer (> 12 hours) than for the monomeric naltrexone control (< 8 h). Serum concentration curves crossed at approximately 7 hours. Additionally, the mean of the peak level concentration (*Cmax*) was significantly lower for the conjugated naltrexone (Table 14). Further, the mean time to peak concentration (*Tmax*) was significantly longer for the polyserine-naltrexone conjugate (Table 14). The mean AUC of the polyserine-naltrexone conjugate was approximately 75% of the naltrexone mean AUC (Table 5). Statistically the mean AUCs were not significantly different ($P < 0.05$). Serum levels of rats fed one-half-dose (1.8 mg) at time zero and at 6.5 hours were compared to those of rats fed polyserine-naltrexone conjugate. Concentration levels remained elevated for the conjugate past those for the second naltrexone dose, with the curves crossing at approximately 2.5 hours and again at approximately 11 hours (double cross-over of the serum concentration curves).

- Table 14. Mean Pharmacokinetic Parameters of Polyserine-Naltrexone vs. Naltrexone -

Dosage Form	*Cmax* +/- SD (ng/ml)	*Tmax* +/- SD (hours)	AUC 0-24h +/- SD (ng h/ml)
Polyserine-naltrexone	38.2 +/- 11.9	7.3 +/- 3.1	356 +/- 66
Naltrexone	124.5 +/- 16.6	0.75 +/- 0.5	477 +/- 183

X:C - *In Situ* Performance of Polyserine-Naltrexone - Caco-2 Cell Digestion

[310] Polyserine-naltrexone conjugates BB-272 and BB-301 were incubated with monolayers of Caco-2 cells for 4 hours in phosphate buffered saline. Buffer was removed from the monlayers and concentrated on SP-18 columns. Concentrated samples were analyzed for the presence of naltexone by reverse phase HPLC. Each Polyserine-naltrexone conjugate showed significant release of free naltrexone from the polymer conjugate in three separate samples. In conclusion, Caco-2 cellular enzymes affected release of naltrexone from Polyserine-naltrexone conjugates BB-272 and BB-301. Release of carbonate linked drug from a conjugate by intestinal cellular enzymes affords a mechanism for drug absorption following oral administration.

X:D - Treatment of Polyserine-naltrexone conjugates with intestinal enzymes

[311] Polyserine-naltrexone (BB-272 and BB-301) were treated with enzymes found in the stomach and lumen of the small intestines. The enzymes tested, which included pepsin, pancreatic lipase, and pancreatin were ineffective in releasing naltrexone from the polyserine-naltrexone conjugates. Other enzymes, including protease and amidase, also did not affect drug release. These results suggest that polyserine-naltrexone is resistant to enzymes found in the stomach and lumen of the intestine.

X:E - Conclusion

[312] In conclusion, conjugation of naltrexone to a polymer of serine via carbonate linkage comprised a pharmaceutical composition that afforded extended release when administered orally. The said conjugates were resistant to a number of

enzymes found in the luminal fluids of the intestinal tract. In contrast, incubation of the compositions with Caco-2 human intestinal epithelial cells affected release of naltrexone. In a specific embodiment of the invention, pharmaceutical compositions comprised of a drug covalently bound to a carrier that are resistant to luminal enzymes and depend on intestinal cell associated enzymes for drug release afford extended release characteristics to the bound drug.**XI. *In vivo* performance of polyglutamic acid-azidothymidine (AZT) conjugate (ester linked)**

XI. *In vivo* performance of polyglutamic acid-azidothymidine (AZT) conjugate (ester linked)

XI:A - *In vivo* performance of polyglutamic acid-azidothymidine (AZT) conjugate

(lot no. TM-113, 41% AZT content)

[313] Polyglutamic acid-AZT conjugates were tested in male Sprague-Dawley rats (~ 250 g). Defined doses were delivered orally in sodium bicarbonate solution containing polyglutamic acid-AZT conjugates or AZT.

[314] Content of AZT in the conjugate TM-113 was estimated to be 41% as based on UV spectrophotometric assay. Polyglutamic acid-AZT conjugate was given to five rats at a dose containing 15 mg/kg of AZT. Doses of AZT equivalent to the AZT contained in polyglutamic acid-AZT (TM-113) were also given to five rats.

[315] Doses were delivered orally to rats at time-zero using an intragastric delivery syringe. Plasma was collected at 0.5, 1.5, 3, 5, 8, 12, and 24 hours after delivery of polyglutamic acid-AZT (TM-113) and equivalent AZT dosed rats. Plasma AZT concentrations were determined by ELISA using a commercially available kit (AZT ELISA, Product #400110, Neogen Corporation, Lexington, KY).

Table 15. Plasma Concentrations of Individual Rats Fed Polyglutamic acid-AZT (TM-113) vs. AZT

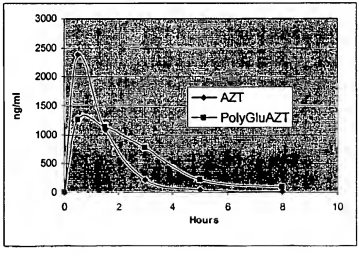
	AZT (ng/ml)					Polylutamic acid-AZT (ng/ml)				
Hours	Rat #1	Rat #2	Rat #3	Rat #4	Rat #5	Rat #1	Rat #2	Rat #3	Rat #4	Rat #5
0	6	4	2	9	14	2	3	3	3	1
0.5	4520	2108	2657	1978	690	1562	779	2084	1015	845

1.5	2275	970	1127	653	418	1775	633	1736	532	1032
3	598	132	200	94	93	1110	367	1713	156	520
5	79	25	78	26	39	46	169	766	35	55
8	16	18	12	4	5	55	224	62	89	3
12	101	38	28	2	6	8	108	118	3	15
24	13	12	4	8	3	2	4	17	2	4

Table 16. Mean Plasma Concentrations of Polyglutamic acid-AZT (TM-113) vs. AZT

Hours	AZT	PolyGlu-AZT (TM-113)
	(ng/ml)	(ng/ml)
0	7 +/- 5	2 +/- 0.9
0.5	2391 +/- 1392	1257 +/- 555
1.5	1089 +/- 718	1142 +/- 591
3	223 +/- 214	773 +/- 634
5	49 +/- 27	214 +/- 313
8	11 +/- 6	87 +/- 83
12	36 +/- 42	50 +/- 57
24	8 +/- 5	6 +/- 6

Plasma concentration curves of polyglutamic acid-AZT (TM-113) vs. AZT



[316] Plasma levels of individual animals are shown in Table 15. Mean plasma levels are shown in Table 16. As shown the figure above, AZT plasma levels spiked at 0.5 hours and rapidly droped by 1.5 hours, whereas levels of AZT for the polyglutamic acid-AZT where significantly lower at 0.5 hours and did not drop off

rapidly by 0.5 hours. Plasma levels of AZT for the polyglutamic acid-AZT conjugate remained elevated longer (> 3 hours) than for the monomeric AZT control (< 3 h). Plasma concentration curves crossed at approximately 1.5 hours. Pharmacokinetic parameters are summarized in Table 17. The mean of the peak levels of concentration (*Cmax*) was significantly lower for the polyglutamic acid-AZT conjugate. Further, the mean time to peak concentration (*Tmax*) was significantly longer for the polyglutamic acid-AZT conjugate. The mean AUC of the polyglutamic acid-AZT (TN-113)conjugate was approximately 124% of the AZT mean AUC.

Table 17. Mean Pharmacokinetic Parameters of polyglutamic acid-AZT (TM-113) vs. AZT

Dosage Form	*Cmax* +/- SD (ng/ml)	*Tmax* +/- SD (hours)	AUC 0-24h +/-SD (ng h/ml)
AZT	2391 +/- 1392	0.5 +/- 0	4,044 +/- 2,689
Polyglutamic acid-AZT (TM-113)	1229 +/- 517	0.9 +/- 0.55	5,000 +/- 3,047

XI:B - *In vivo* performance of polyglutamic acid-azidothymidine (AZT) conjugate (rat model)

(lot no. TM-248, 43% AZT content)

[317] Polyglutamic acid-AZT conjugates were tested in male Sprague-Dawley rats (~ 250 g). Defined doses were delivered orally in sodium bicarbonate solution containing polyglutamic acid-AZT conjugates or AZT.

[318] Content of AZT in the conjugate TM-248 was estimated to be 43 % as based on UV spectrophotometric assay. Polyglutamic acid-AZT conjugate was given to five rats at a dose containing 7.5 mg/kg of AZT. Doses of AZT equivalent to the AZT contained in polyglutamic acid-AZT (TM-248) were also given to five rats.

[319] Doses were delivered orally to rats at time-zero using an oral gavage syringe. Plasma was collected at 0.5, 1, 2, 3, 4, 6, and 9 hours after delivery of polyglutamic acid-AZT (TM-248) and equivalent AZT dosed rats. Plasma AZT

concentrations were determined by ELISA using a commercially available kit (AZT ELISA, Product #400110, Neogen Corporation, Lexington, KY).

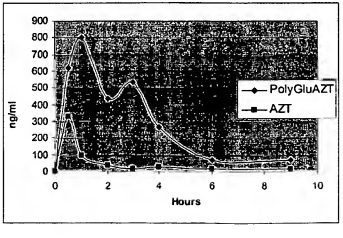
Table 18. Plasma Concentrations of Individual Rats Fed polyglutamic acid-AZT (TM-248) vs. AZT

	AZT(ng/ml)				Polyglutamic acid-AZT (ng/ml)			
Hours	Rat #1	Rat #2	Rat #3	Rat #4	Rat #1	Rat #2	Rat #3	Rat #4
0	0	2	1	0	0	0	1	1
0.5	600	535	175	21	582	1307	277	312
1	303	71	10	1	1111	1365	440	282
2	140	0	4	6	779	664	287	27
3	63	10	1	0	574	965	528	73
4	69	30	5	4	160	569	296	43
6	36	11	9	6	12	190	79	7
9	10	37	14	8	20	192	27	39

Table 19. Mean Plasma Concentrations of Polyglutamic acid-AZT (TM-248) vs. AZT

Hours	AZT (ng/ml)	PolyGlu-AZT (TM-248) (ng/ml)
0	1 +/- 1	1 +/- 1
0.5	333 +/- 280	620 +/- 478
1	96 +/- 141	800 +/- 521
2	38 +/- 68	439 +/- 346
3	19 +/- 30	535 +/- 365
4	27 +/- 30	267 +/- 226
6	16 +/- 14	72 +/- 85
9	17 +/- 13	70 +/- 82

- Plasma concentration curves of polyglutamic acid-AZT (TM-113) vs. AZT -



[320] Plasma levels of individual animals are shown in Table 18. Mean plasma levels are shown in Table 19. As shown the figure above, AZT plasma levels spiked at 0.5 hours and rapidly dropped by 1 hour, whereas levels of AZT for the polyglutamic acid-AZT remained elevated until 4 hours. Pharmacokinetic parameters are summarized in Table 20. *Cmax* of polyglutamic acid-AZT (TM-248) was increased by 149% over AZT. The mean AUC of polyglutamic acid-AZT (TM-248) was increased 598% over AZT. Further, the mean time to peak concentration (*Tmax*) was substantially longer for the polyglutamic acid-AZT conjugate. This example clearly illustrates that both enhanced absorption and sustained release can be afforded to AZT by covalent attachment to a polymer of glutamic acid.

Table 20. Mean Pharmacokinetic Parameters of polyglutamic acid-AZT (TM-248) vs. AZT

Dosage Form	*Cmax* +/- SD (ng/ml)	*Tmax* +/- SD (hours)	AUC 0-24h +/-SD (ng h/ml)
AZT	333 +/- 280	0.5 +/- 0	398 +/- 401
Polyglutamic acid-AZT (TM-248)	829 +/- 491	0.875 +/- .25	2,777 +/- 1,811

What Is Claim Is:

1. A composition comprising:

a carrier peptide that comprises at least one active agent covalently attached to said carrier peptide at the C-terminus, interspersed, side-chain attached, or combination thereof;

wherein said carrier peptide further comprises fewer than 50 amino acids; and

wherein said composition is in a form suitable for release of said active agent into the bloodstream.

2. The composition of claim 1, wherein said carrier peptide is a single amino acid.

3. The composition of claim 1, wherein said carrier peptide comprises dipetide.

4. The composition of claim 3, wherein said dipeptide further comprises at least one of glutamine, glutamic acid, aspartic acid, serine, lysine, cysteine, threonine, tyrosine, asparagine, or arginine.

5. The composition of claim 1, wherein said carrier peptide is a tripeptide.

6. The composition of claim 5, wherein said tripeptide further comprises at least one of glutamine, aspartic acid, glutamic acid, serine, lysine, cysteine, threonine, tyrosine, asparagine, or arginine.

7. The composition of claim 1, wherein said carrier peptide has a length between four and eight amino acids.

8. The composition of claim 1, wherein said carrier peptide has a length between four and 15 amino acids.

9. The composition of claim 1 wherein said carrier peptide has a length between nine and 50 amino acids.

10. The composition of claim 1, wherein said active agent is selected from Table 2.

11. The composition of claim 1, comprising multiple active agents.

12. The composition of claim 1, wherein said carrier peptide active agent composition is selected from the group of active agent bound to -Ser-Ser, -PolySer, -Lys, -Glu-Glu, Asp-Asp, Asp-Asp-Asp, Asp-Asp-Glu, Asp-Asp-Ser, Asp-Asp-Lys, Asp-Asp-Cys, Ala-Glu, Ala-Ser, Ala-Asp, Ala-Asn, Ala-Thr, Ala-Arg, Ala-Cys, Ala-Gln, Ala-Tyr, LeuGlu, Leu-Ser, Leu-Asp, Leu-Asn, Leu-Thr, Leu-Arg, Leu-Cys, Leu-Gln, Leu-Tyr, PheGlu, Phe-Ser, Phe-Asp, Phe-Asn, Phe-Thr, Phe-Arg, Phe-Cys, Phe-Gln, Phe-Tyr, Val-Glu, Val-Ser, Val-Asp, Val-Asn, Val-Thr, Val-Arg, Val-Cys, Val-Gln, Val-Tyr.

13. The composition of claim 1, wherein said active agent is an amino acid active agent and is covalently interspersed in said carrier peptide.

14. The composition of claim 1, further comprising said carrier peptide covalently attached to a fatty acid.

15. The composition of claim 1, wherein said carrier peptide is covalently attached to PEG.

16. The composition of claim 1, wherein said carrier peptide is covalently attached to cyclodextrin.

17. The composition of claim 1, wherein said carrier peptide is non-covalently associated with cyclodextrin.

18. The composition of claim 1, wherein a polymer formulation affords sustained release of the active agent.

19. The composition of claim 1, wherein said carrier peptide enhances absorption.

20. The composition of claim 1, wherein said carrier peptide is a homopolymer or a heteropolymer.

21. The composition of claim 23, wherein said carrier peptide is a homopolymer comprising naturally occurring amino acids.

22. The composition of claim 23, wherein said carrier peptide is a homopolymer comprising synthetic amino acids.

23. The composition of claim 23, wherein said carrier peptide is a heteropolymer comprising two or more naturally occurring amino acids.

24. The composition of claim 23, wherein said carrier peptide is a heteropolymer comprising two or more synthetic amino acids.

25. The composition of claim 23, wherein said carrier peptide is a heteropolymer comprising one or more naturally occurring amino acids and one or more synthetic amino acids.

26. The composition of claim 1, wherein said composition further comprises at least one adjuvant attached to said carrier peptide.

27. The composition of claim 29, wherein said adjuvant enhances absorption.

28. The composition of claim 29, wherein said adjuvant enhances bioadhesive properties.

29. The composition of claim 31, wherein said bioadhesive property of said adjuvant imparts sustained release pharmacokinetics of the active agents in the alimentary tract.

30. The composition of claim 29, wherein said adjuvant targets delivery of the carrier peptide to specific locations in the alimentary tract, specific cells in the systemic circulation or to specific enzymes in the intestinal lumen or brush border membrane.

31. The composition of claim 29, wherein said adjuvant is the peptide itself.

32. The composition of claim 1, wherein said carrier peptide is in a form which enhances the solubility of the active agent in aqueous or organic solvents.

33. The composition of claim 35, wherein said solubility allows even dispersion in polymer formulations.

34. The composition of claim 1, wherein said carrier peptide is in a form which affords sustained release kinetics of absorption to the parent drug.

35. The composition of claim 1, wherein said composition is in a form designed to allow release of said active agent according to zero order kinetics.

36. The composition of claim 1, wherein said composition is in a form that enhances therapeutic efficacy of said active agent by decreasing toxicity of said active agent, decreasing clearance rate of said active agent, increasing steady state concentration of said active agent, maintaining therapeutic levels for an extended period of the active agent, or avoiding drops in the effective level of said active agent or combinations thereof.

37. The composition of claim 1, wherein said composition is in a form that enhances the *in vivo* stability of said active agent.

38. The composition of claim 1, wherein said composition is in a form that enhances the *in vitro* stability of said active agent.

39. A composition comprising:

a carrier peptide that comprises at least one active agent covalently attached to said carrier peptide at the N-terminus;

wherein said carrier peptide is not individually Glu, Tyr, Ala, Met, or Gly-Gly and has a length less than 50 amino acids; and

wherein said composition is in a form suitable for release of said active agent into the bloodstream.

40. A composition comprising:

a carrier peptide that comprises at least one active agent covalently attached to said carrier peptide;

wherein said carrier peptide has a length between 1 and 500 amino acids; and

wherein said composition is in a form suitable for release of said active agent into the bloodstream from the alimentary tract.

41. A composition comprising:

a carrier peptide with a length between one and 50 amino acids;

at least one active agent covalently bound to said carrier peptide; and

wherein said composition is in a form suitable for modulation of the pharmacological effect of said composition in the small intestine.

42. A composition comprising:

a carrier peptide with a length between three and nine amino acids; and

at least one active agent covalently bound to said carrier peptide.

43. A composition comprising: a carrier peptide that comprises at least one active agent covalently attached to said carrier peptide; wherein said carrier peptide is a single amino acid with the proviso that the amino acid is not alanine, glutamine, tyrosine, methionine.

44. A composition comprising: a carrier peptide and at least one active agent covalently attached to said carrier peptide; wherein said carrier peptide is a dipeptide with the proviso that the dipeptide is not gly-gly.

45. A composition comprising: a carrier peptide that comprises at least one active agent covalently attached to said carrier peptide with the proviso that said active agent is not salicylic acid; and wherein said carrier peptide is in a form suitable for release of said active agent into the bloodstream.

46. A composition comprising:
a carrier peptide wherein said carrier peptide has a length between one and 50 amino acids and is in a form suitable for pharmacological effect in the small intestine of a subject; and
at least one active agent covalently bound to said carrier peptide wherein said active agent is released into the bloodstream.

47. A composition comprising:
a carrier peptide wherein said carrier peptide has a length between one and 50 amino acids and is in a form suitable for release of said active agent into the bloodstream; and
at least two active agents covalently bound to said carrier peptide.

48. A composition comprising:

a carrier peptide wherein said carrier peptide has a length between one and 50 amino acids in a form wherein said active agent is delivered into systemic circulation; and

an active agent covalently bound to said carrier peptide.

49. A composition comprising:

a carrier peptide wherein said carrier peptide has a length between one and 50 amino acids in a form which converts the mechanism of drug adsorption from passive to active uptake; and

an active agent covalently bound to said carrier peptide.

50. A method of treating a disease or disorder comprising administering the composition of claim 1.

51. A method of treating disorders, said method comprising administering to a subject a composition comprising:

a carrier peptide; and

at least one active agent covalently attached to said carrier peptide wherein said carrier peptide comprises fewer than 50 amino acids in length; and

wherein said carrier peptide is in a form suitable for delivery into the bloodstream.

52. A composition comprising at least one active agent covalently bound to PEG in a form suitable for modulation in the alimentary tract.

53. A composition comprising at least one active agent covalently bound to cyclodextrin in a form suitable for modulation in the alimentary tract.

54. A composition comprising at least one active agent covalently bound to fatty acid in a form suitable for modulation in the alimentary tract.

55. The composition of claim 48, wherein said composition further comprises an adjuvant non-covalently bound to said carrier peptide.

56. The composition of claim 48, wherein the parent drug but not the conjugate-drug composition reaches the systemic circulation.

AMENDED CLAIMS
received by the International Bureau on 20 October 2003 (20.10.2003)
original claims 21-25, 27-31 are replaced.

12. The composition of claim 1, wherein said carrier peptide active agent composition is selected from the group of active agent bound to -Ser-Ser, -PolySer, -Lys, -Glu-Glu, Asp-Asp, Asp-Asp-Asp, Asp-Asp-Glu, Asp-Asp-Ser, Asp-Asp-Lys, Asp-Asp-Cys, Ala-Glu, Ala-Ser, Ala-Asp, Ala-Asn, Ala-Thr, Ala-Arg, Ala-Cys, Ala-Gln, Ala-Tyr, LeuGlu, Leu-Ser, Leu-Asp, Leu-Asn, Leu-Thr, Leu-Arg, Leu-Cys, Leu-Gln, Leu-Tyr, PheGlu, Phe-Ser, Phe-Asp, Phe-Asn, Phe-Thr, Phe-Arg, Phe-Cys, Phe-Gln, Phe-Tyr, Val-Glu, Val-Ser, Val-Asp, Val-Asn, Val-Thr, Val-Arg, Val-Cys, Val-Gln, Val-Tyr.

13. The composition of claim 1, wherein said active agent is an amino acid active agent and is covalently interspersed in said carrier peptide.

14. The composition of claim 1, further comprising said carrier peptide covalently attached to a fatty acid.

15. The composition of claim 1, wherein said carrier peptide is covalently attached to PEG.

16. The composition of claim 1, wherein said carrier peptide is covalently attached to cyclodextrin.

17. The composition of claim 1, wherein said carrier peptide is non-covalently associated with cyclodextrin.

18. The composition of claim 1, wherein a polymer formulation affords sustained release of the active agent.

19. The composition of claim 1, wherein said carrier peptide enhances absorption.

20. The composition of claim 1, wherein said carrier peptide is a homopolymer or a heteropolymer.

21. The composition of claim 20, wherein said carrier peptide is a homopolymer comprising naturally occurring amino acids.

22. The composition of claim 20, wherein said carrier peptide is a homopolymer comprising synthetic amino acids.

23. The composition of claim 20, wherein said carrier peptide is a heteropolymer comprising two or more naturally occurring amino acids.

24. The composition of claim 20, wherein said carrier peptide is a heteropolymer comprising two or more synthetic amino acids.

25. The composition of claim 20, wherein said carrier peptide is a heteropolymer comprising one or more naturally occurring amino acids and one or more synthetic amino acids.

26. The composition of claim 1, wherein said composition further comprises at least one adjuvant attached to said carrier peptide.

27. The composition of claim 26, wherein said adjuvant enhances absorption.

28. The composition of claim 26, wherein said adjuvant enhances bioadhesive properties.

29. The composition of claim 28, wherein said bioadhesive property of said adjuvant imparts sustained release pharmacokinetics of the active agents in the alimentary tract.

30. The composition of claim 26, wherein said adjuvant targets delivery of the carrier peptide to specific locations in the alimentary tract, specific cells in the systemic circulation or to specific enzymes in the intestinal lumen or brush border membrane.

31. The composition of claim 26, wherein said adjuvant is the peptide itself.

32. The composition of claim 1, wherein said carrier peptide is in a form which enhances the solubility of the active agent in aqueous or organic solvents.

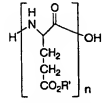
33. The composition of claim 32, wherein said solubility allows even dispersion in polymer formulations.

34. The composition of claim 1, wherein said carrier peptide is in a form which affords sustained release kinetics of absorption to the parent drug.

35. The composition of claim 1, wherein said composition is in a form designed to allow release of said active agent according to zero order kinetics.

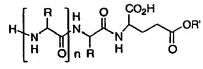
Figure 1:

Mechanism of Alcohol Drug from Glutamic Acid Dimer Scheme



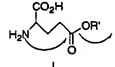
Poly[γ-alkylglutamate]

OR



Drug-Peptide Conjugate

Intestinal Peptidases



+ other amino acid byproducts

Pyroglutamic Acid

R'OH

Alcohol Drug

R' = Radical Moiety attached to alcohol functionality on drug
R = Side chain of amino acid or peptide

Figure 2: *In situ* Digestion of Polythroid in Intestinal Epithelial Cell Cultures

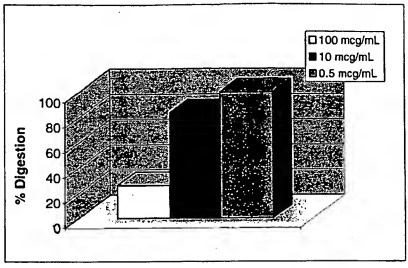


Figure 3: Basolateral T4 Concentrations

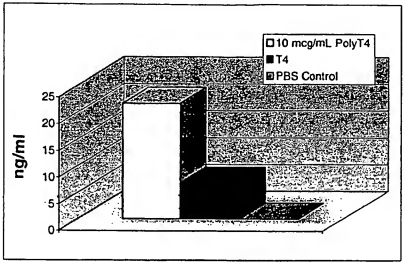
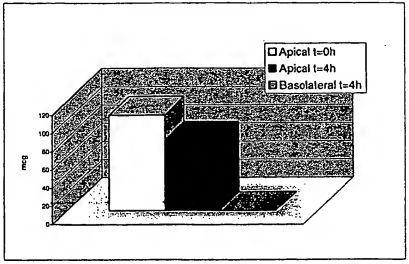


Figure 4: Polythroid concentration

Apical vs. Basolateral



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/17009

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claim Nos.: 29, 30, and 31
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/17009

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 38/02, 38/03
US CL : 514/2; 530/345
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/426, 436, 457, 460, 468, 486, 499; 514/2; 530/345, 405, 409

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,183,883 A (TANAKA ET AL) 02 February 1993 (02/02/93), see entire document, especially the Abstract.	53
X	US 5,534,496 A (LEE ET AL) 09 July 1996 (09/07/96), see entire document, especially the abstract, column 2, line 55 - column 3, line 35, column 4, lines 17-25, column 8, line 29 - column 9, line 6, claims 1-8.	1, 3, 5, 7, 8, 10, 17-20, 23, 26-28, 32-42, 44-46, 48-51, 55, 56
X	US 5,891,459 A (COOKE ET AL) 06 April 1999 (06/04/99), see entire document, especially the Abstract, column 7, line 54 - column 8, line 4, column 9, line 61 - column 10, line17.	1-13, 18-21, 23, 26-28, 32-51, 56
X	US 5,910,569 A (LATHAM ET AL) 08 June 1999 (08/06/99), see entire document, especially the Abstract, column 1, lines 26-62, column 2, lines 51-52, column 4, lines 38-46, column 5, lines 8-14, column 8, line 59 - column 9, line 15, column 14, lines 28-33.	1, 7-11, 13, 1828, 32-42, 45-51, 56
X	US 6,309,633 B1 (EKWURIBE ET AL) 30 October 2001 (30/10/01), see entire document, especially the Abstract, column 4, line 65 - column 5, line 60.	52, 54

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 July 2003 (24.07.2003)	03 SEP 2003
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703)305-3230	Authorized officer Jeffrey E. Russel Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

PCT/US03/17009

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2001/0031873 A1 (GREENWALD ET AL) 18 October 2001 (18/10/01), see entire document, especially the Abstract, paragraphs 0018, 0019, 0037, 0139-0142.	1-12, 14, 15, 18-28, 32-38, 40-46, 48-52, 54, 56
X	WO 02/34237 A1 (NEW RIVER PHARMACEUTICALS, INC.) 02 May 2002 (02/05/02), see entire document, especially claims 1-40.	1-28, 32-56

INTERNATIONAL SEARCH REPORT

PCT/US03/17009

Continuation of B. FIELDS SEARCHED Item 3:
EAST, DIALOG
search terms: conjugate, peg, cyclodextrin, fatty acid